Solving Poison Control Center intoxication cases using Time-of-Flight Mass Spectrometry

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Outline

Toxic Substance Identification

Time-of-Flight Mass Spectrometry

Targeted and Non-targeted Drug Screening
Using TOF LC-MS

Cases

(Potential Applications to Environmental Biomonitoring)
Emergency Intoxications

Annual ED visits: 124 M
Emergency intoxications: 0.9 M
Toxicology Screens ordered: 3.7 M

Drugs Most Commonly Reported
- Ibuprofen (1-17M)
- Acetaminophen/ Hydrocodone (2-14.5M)
- Morphine (6-7M)
- Hydromorphone (8-5.7M)
- Acetaminophen/ Oxycodone (9-5.3M)
- Diphenhydramine (13-3.6M)

Drugs Commonly Detected: Ethyl alcohol, Cocaine, THC, Heroin, Stimulants, PCP, MDMA
Identifying Toxic Substance in Patients

Toxidromes (T, BP, HR, RR, mental status)

Initial Diagnosis

Therapeutic Intervention

Order Tests (e.g. DAU)

Confirm/ Revise Diagnosis

Patient Disposition
Identifying Toxic Substance in Patients

Toxidromes

Initial Diagnosis

Order Tests (e.g. DAU)

Confirm/ Revise Diagnosis

Patient Disposition

Therapeutic Intervention
Identifying Toxic Substance in Patients

Challenges in Emergency Intoxication Testing

- lack of serum comprehensive drug tests for emergency testing
- long turnaround times of analysis (1-2 days)
- lack of information on the clinical utility of laboratory test results
- some emergency cases are quite complex that targeted screening may not be sufficient
Most Common Methods Used in Toxic Substance Identification

Immunoassay

Chromatography- HPLC, GC

LC-Mass Spectrometry/ GC-Mass Spectrometry
Immunoassay

Use of antibody to identify a structural motif in a compound or class of compounds

Targeted screening- antibody is raised against a specific compound or class of compounds

Specificity is not always good; cross-reactivity of compounds with similar structural motif to the targeted compound
Chromatography

Separation of compounds according to differences in polarity

Compounds are detected according to their chemical property (e.g. UV absorbance)

Order of elution in a NON-POLAR stationary phase:

1 > 2 > 3

Limited specificity in complex biological matrices - urine and serum
LC (GC)- Mass Spectrometry

Ionization
ION SOURCE
Form ions (charged molecules)

Mass Sorting (filtering)
MASS ANALYZER
Sort ions by mass (m/z)

Detection
ION DETECTOR
Detect ions

Inlet

HPLC/ GC

- Solid
- Liquid
- Vapor

Mass Spectrum

216.0790
(M+H)+

Counts vs. Mass-to-Charge (m/z)

Cpd 8: 2C-C: +ESI Scan (3.259-3.295 min, 7 scans) Frag=125.0V AS1-DF10000-101810-2.5uL-10...
LC (GC)- Mass Spectrometry

Modes by which MS identify molecules

1. Unique fragmentation pattern

   - Molecule 1: 122 → 92 + 33
   - Molecule 2: 122 → 78 + 47

2. Accurate Mass
Q1. Ions of interest are selected (parent ions)
Q2. Fragmented into smaller product ions
Q3. Product ions separated by mass (m) to charge (z) ratio (m/z)
Compounds are characterized by their specific fragmentation patterns (specific mass transitions)- e.g. 339→177 and 339→139

Compounds are identified by building a database of specific transitions

Method has high specificity and sensitivity

Cannot be used for non-targeted screening
Time-of-Flight Mass Spectrometry (MS-TOF)

Time of flight of ions measured
TOF of ion is proportional to its m/z
High mass accuracy in the sub-2 ppm
Principle: If ions are accelerated with the same potential at a fixed point and a fixed initial time and are allowed to drift, the ions will separate according to their mass to charge ratios.
Time-of-flight mass analyzer

The ions enter the flight tube with the lighter ions travelling faster than the heavier ions to the detector.
The lighter ions strike the detector before the heavier ions. This “time of flight” (TOF) can be converted to mass.
TOF reflectron allows for higher accuracy

Ions are sent back down part of the flight tube. This device corrects for ions of same mass but different energies.
Mass Accuracy

Accuracy is measured as mass error (in ppm)

Mass error (ppm) = \[ \frac{|MW_{\text{meas}} - MW_{\text{theo}}|}{MW_{\text{theo}}} \cdot 1 \times 10^6 \]

\[ = \frac{|250.0005 - 250.0000|}{250.0000} \cdot 1 \times 10^6 \]

\[ = 2 \text{ ppm} \]

2 ppm accuracy translates to:

<table>
<thead>
<tr>
<th>MW</th>
<th>amu error</th>
</tr>
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<tbody>
<tr>
<td>100</td>
<td>0.0002</td>
</tr>
<tr>
<td>200</td>
<td>0.0004</td>
</tr>
<tr>
<td>300</td>
<td>0.0006</td>
</tr>
<tr>
<td>400</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
Mass Accuracy

Morphine: $C_{17}H_{18}NO_3$

285.1365

Pentazocine: $C_{19}H_{27}NO$

285.2093

For M/Z = 285.1365

At 10ppm accuracy  5 possible formula
At  5ppm accuracy   $C_{17}H_{18}NO_3, C_{15}H_{26}N_4O_2, C_{20}H_{16}N_2$
At  3ppm accuracy   $C_{17}H_{18}NO_3$
Qualitative Analysis

Agilent 6230 TOF

Total Ion Chromatogram

Targeted Screening
• Database search
• Formula search

Non-Targeted Analysis
# Targeted Screening by Database Search

Library of Compounds with established RT is created (DATABASE)
Database is used as reference in searching for unknowns in a sample
Search TIC for formula and RT time matches in the library
RT match within 0.15 min
Mass accuracy < 10ppm
Target Score ≥ 70

<table>
<thead>
<tr>
<th>Name</th>
<th>RT</th>
<th>RT (Tgt)</th>
<th>RT Diff (Tgt)</th>
<th>Mass</th>
<th>Diff (Tgt, ppm)</th>
<th>Formula (Tgt)</th>
<th>Score (Tgt)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>2.597</td>
<td>2.643</td>
<td>-0.046</td>
<td>135.1058</td>
<td>7.39</td>
<td>C9H13N</td>
<td>87.73</td>
<td>189303</td>
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<tr>
<td>Benzoylecgonine</td>
<td>3.065</td>
<td>3.12</td>
<td>-0.055</td>
<td>289.1311</td>
<td>-1.18</td>
<td>C16H19NO4</td>
<td>87.45</td>
<td>870459</td>
</tr>
<tr>
<td>Benzylpiperazine</td>
<td>2.27</td>
<td>2.275</td>
<td>-0.005</td>
<td>176.1329</td>
<td>8.67</td>
<td>C11H16N2</td>
<td>89.27</td>
<td>1164833</td>
</tr>
<tr>
<td>Buprenorphined-4</td>
<td>4.093</td>
<td>4.184</td>
<td>-0.091</td>
<td>471.3273</td>
<td>-2.91</td>
<td>C29H37D4NO4</td>
<td>73.11</td>
<td>751809</td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>4.493</td>
<td>4.582</td>
<td>-0.089</td>
<td>260.173</td>
<td>2.48</td>
<td>C12H24N2O4</td>
<td>78.43</td>
<td>362501</td>
</tr>
<tr>
<td>Cocaine</td>
<td>3.174</td>
<td>3.249</td>
<td>-0.075</td>
<td>303.1469</td>
<td>0.53</td>
<td>C17H21NO4</td>
<td>82.05</td>
<td>1963967</td>
</tr>
</tbody>
</table>
Panels Available for Targeted Screening

Seizure Panel (44 drugs, serum)

Drugs-of-Abuse Panel (213 drugs, serum)
  Phenylethylamines (41), Stimulants (9), Psychotropic alkaloids (10), Opioids (28), Benzodiazepines (32), Barbiturates (16), Antidepressants (24), Sedatives/Hypnotics (10), Anesthetics (10), Antihistamines (14), Analgesics (12), Muscle Relaxants (7)

Comprehensive Drug Screen (319, serum)
  DOA panel plus Anticonvulsants (23), Antipsychotics (14), Cardiovascular (44), Respiratory (8), Antidiabetics (8), Anorectics (9)

Synthetic Cannabinoids (33 compds, serum, urine, pill)

Herbal Bioactive Markers (serum, urine, pill)
  22 compounds from 20 of the most commonly used herbal medications
Targeted Screening by Formula Search

JWH-018 is a synthetic cannabinoid commonly used to lace herbal incense.

JWH-018 is metabolized rapidly and usually not detected in serum and urine.

Its metabolites have been detected in urine.

Predicted metabolites:

- Hydroxylation: $C_{24}H_{24}NO_2$
- N-desalkylation: $C_{19}H_{12}NO$
- Hydroxylation/N-desalkylation: $C_{19}H_{12}NO_2$

JWH-018
$C_{24}H_{23}NO$
Non-Targeted Analysis: Molecular Feature Extractor

How MFE works -

• Map signal in the 3-dim. space in time and mass at the MS level
• Remove areas which only contain noise and NO signals
• Identify all mass signals with a common RT (narrow time window)
• Combine mass signals with common RT and chemical relation (isotope adduct, dimer, higher charge state) → Molecular Feature or Compound

![Diagrams and tables related to MFE process]
Non-Targeted Analysis: Qualitative Analysis

<table>
<thead>
<tr>
<th>Compound List</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Show/Hide</td>
<td>Cpd</td>
<td>Name</td>
<td>Score (MFG)</td>
<td>RT</td>
<td>Mass</td>
<td>Diff (MFG, ppm)</td>
<td>Formula (MFG)</td>
</tr>
<tr>
<td>---------------</td>
<td>-----</td>
<td>------------</td>
<td>-------------</td>
<td>-----</td>
<td>------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>0.515</td>
<td>150.1403</td>
<td>3.49</td>
<td>C11 H18</td>
</tr>
</tbody>
</table>

- **Compound List**
  - **Pyrrolidinopropiophenone**
    - Score (MFG): 86.75
    - RT: 0.876
    - Mass: 203.1298
    - Diff (MFG, ppm): 6.16
    - Formula (MFG): C13 H17 N O
    - Ions: 3
    - Height: 995274

- **Compound List**
  - **Oxycodone**
    - Score (MFG): 93.05
    - RT: 3.653
    - Mass: 315.149
    - Diff (MFG, ppm): -6.07
    - Formula (MFG): C18 H21 N O4
    - Ions: 4
    - Height: 1087365

- **Compound List**
  - **Orthocaine**
    - Score (MFG): 97.77
    - RT: 1.177
    - Mass: 167.0582
    - Diff (MFG, ppm): 0.18
    - Formula (MFG): C8 H9 N O3
    - Ions: 4
    - Height: 1242958

- **Compound List**
  - **Nor-triptiline**
    - Score (MFG): 94.44
    - RT: 3.721
    - Mass: 263.1689
    - Diff (MFG, ppm): -5.78
    - Formula (MFG): C19 H21 N
    - Ions: 3
    - Height: 1071428

- **Compound List**
  - **m-Hydroxybenzoylecgonine**
    - Score (MFG): 90.76
    - RT: 2.616
    - Mass: 305.1278
    - Diff (MFG, ppm): -4.91
    - Formula (MFG): C16 H19 N O5
    - Ions: 4
    - Height: 2396293

- **Compound List**
  - **Methadone**
    - Score (MFG): 99.65
    - RT: 0.963
    - Mass: 275.1402
    - Diff (MFG, ppm): -1.05
    - Formula (MFG): C11 H22 N O3 P
    - Ions: 3
    - Height: 960282

- **Compound List**
  - **Levamisole**
    - Score (MFG): 97.15
    - RT: 2.439
    - Mass: 204.073
    - Diff (MFG, ppm): -4.31
    - Formula (MFG): C11 H12 N2 S
    - Ions: 4
    - Height: 1392675

- **Compound List**
  - **Isamfazone**
    - Score (MFG): 79.71
    - RT: 4.485
    - Mass: 361.1765
    - Diff (MFG, ppm): 6.94
    - Formula (MFG): C22 H23 N3 O2
    - Ions: 4
    - Height: 2286808
What can be analyzed using the TOF?

Targeted and Non-Targeted Screening of Small Molecules
Small Molecules: 75-3000 amu (100-1000 amu)

- Most Organic Drugs and metabolites, Herbal and Food Supplements’ bioactive compounds and adulterants, Small molecule biomarkers, Non-proteinaceous hormones, Small peptides, Steroids, Fatty acids, Fat and Cholesterol derivatives, Nucleotides and derivatives, Sugars and small oligosaccharides (~10mer), Pesticides, Small Organic Environmental Pollutants

Samples: Serum, Urine, CSF, Breast milk, Other Body Fluids (Saliva, Sweat etc.), Tissue extracts, Plant extracts, Cell lysates, Pills

Qualitative Data, Semi-quantitative, Quantitative
TAT for LC-MS/TOF run: 8-12 min   Sample Prep: 30-60min

We can develop methods of analysis for specific groups of compds

We can’t analyze proteins, nucleic acids, polysaccharides, polymers, metals, gaseous compds, inorganic salts
CASES
PCC-centric Operation of a Regional Toxicology Lab: The NACB Paradigm
CASE

• 45yo F with multiple seizures, later became delirious
• Family claims patient has been taking an herbal sleep aid supplement and was suspicious that something in the herbal supplement caused the condition
• Attending physician consulted PCC regarding potential herbal supplement O/D

Red Herring
Nyquil-induced seizure?

Case 1
27yo F with hx of EtOH abuse (9 days s/p cessation)
Reported taking 6 tabs generic Nyquil with BF and had seizure 2 hrs post-ingestion (witnessed BF seizing first); tachycardic, diaphoretic
Negative APAP (at 14th hr), amphetamines, cocaine, benzos, opitates
Received IVF and discharged 16hrs post-ingestion

Case 2
31yo M with hx of EtOH abuse (9 days s/p cessation)
Reported taking 6 tabs generic Nyquil with GF and had seizure 2 hrs post-ingestion; tachycardic, diaphoretic
Negative APAP (at 14th hr), amphetamines, cocaine, benzos, opitates
Received IVF and discharged 16hrs post-ingestion
Nyquil-induced seizure?

Seizure Panel Drugs (44)

Anesthetic - Lidocaine, Phencyclidine
Anticonvulsant - Carbamazepine, Topiramate
Antidepressant - Amitriptyline, Desipramine, Doxepin, Imipramine, Nortriptyline, Protriptyline, Trimipramine

- Bupropion, Citalopram, Fluoxetine, Paroxetine, Sertraline, Venlafaxine

Antihistamine - Diphenhydramine, Doxylamine, Hydroxyzine
Antipsychotic - Clozapine, Lamotrigine, Olanzapine, Quetiapine
Cardiovascular - Propranolol
Muscle Relaxant - Carisoprodol
Opioid Analgesic - Meperidine, Propoxyphene, Tramadol
Psychotrop Alkaloid - Benzoylecgonine, Cocaine
Sedative/Hypnotic - Dextromethorphan
Stimulant - Amphetamine, Benzylpiperazine, Caffeine, MDA, MDE, MDMA, Methamphetamine, Methylphenidate, PMA, Trifluoromethylphenylpiperazine
Tuberculostatic - Isoniazid

Drugs chosen according to three surveys conducted by the California Poison Control Center on drugs commonly reported to cause drug-induced seizures in the San Francisco Bay Area
Serum and urine samples were run on seizure panel; searches for pseudoephedrine, phenylephrine and acetaminophen (reported Nyquil components) were also done.

**Lab Results**
No matches for dextromethorphan, pseudoephedrine, phenylephrine, doxylamine and acetaminophen found in the urine and serum sample
BUT
Both patients have tramadol in their serum and urine

**Patient 1**  Serum Tramadol= 2974 ng/mL
**Patient 2**  Serum Tramadol= 1676 ng/mL
Serum Hydroxyzine= 91.25 ng/mL

<table>
<thead>
<tr>
<th></th>
<th>Tramadol</th>
<th>Hydroxyzine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic level</strong></td>
<td>100-800 ng/mL</td>
<td>50-90 ng/mL</td>
</tr>
<tr>
<td><strong>Toxic level</strong></td>
<td>1000 ng/mL</td>
<td>100 ng/mL</td>
</tr>
<tr>
<td><strong>Lethal level</strong></td>
<td>2000 ng/mL</td>
<td></td>
</tr>
</tbody>
</table>
Case

• 15 yo M with vfib arrest after smoking marijuana and ingestion of unknown green pill with puma imprint on it.
• Patient was shocked out of vfib and was intubated and sedated but had very labile blood pressure, intermittently requiring pressors and antihypertensive meds.
• On HD 4, he was extubated, off pressors, and was mentally oriented

• Patient’s serum and urine samples, green pill and two carrying tubes were sent to SFGH for analysis.

Lab Consult
Identify active ingredient of unknown green pill, compounds on tubes and match them with serum and urine samples
# Unknown Green Pill

## Sample Analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>MDMA (Ecstasy)</th>
<th>MDA</th>
<th>MDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, 2/20</td>
<td>87 ng/mL</td>
<td>18.82 ng/mL</td>
<td>61 ng/mL</td>
</tr>
<tr>
<td>Serum, 2/26</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Urine, 2/22</td>
<td>395 ng/mL</td>
<td>123.3 ng/mL</td>
<td>46 ng/mL</td>
</tr>
<tr>
<td>Green Pill</td>
<td>114 mg/125mg</td>
<td>ND</td>
<td>4.38 mg/125mg</td>
</tr>
<tr>
<td>Tube 1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tube 2</td>
<td>474 ng/mL</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Ecstasy Pill = 80-120 mg MDMA  
MDMA Recreational Level (Serum): 100-250ng/mL
## Unknown Green Pill

### Sample Analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Potential Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, 2/20</td>
<td>Trimethoxyamphetamine</td>
</tr>
<tr>
<td>Urine, 2/22</td>
<td>Trimethoxyamphetamine, Trimethoxymethamphetamine</td>
</tr>
<tr>
<td>Green Pill</td>
<td>Trimethoxyamphetamine, Trimethoxymethamphetamine, Methylenedioxy-2-methoxyamphetamine</td>
</tr>
<tr>
<td>Tube 1</td>
<td>Codeine, Trimethoxyamphetamine, Trimethoxymethamphetamine</td>
</tr>
<tr>
<td>Tube 2</td>
<td>Trimethoxyamphetamine</td>
</tr>
</tbody>
</table>
Case of Mistaken Identity

Case One
57yo non-diabetic male with hx of benzodiazepine abuse.
Fingerstick glucose = 41mg/dL
Patient was administered with naloxone and continuous glucose infusion.
Admission glucose = 33 mg/dL
Octeotride and glucagon given, ED doctors were suspicious of sulfonylurea intoxication

On HD2, patient’s glucose rebounded to normal range without supplemental glucose, extubated and his mental status became clear.

Case Two
48yo F found unresponsive by son at home; GCS=5, glucose was undetectable as per paramedics report. Administration of one ampule of glucose, patient’s GCS improved to 7.
Upon ED arrival, patient became alert upon administration of second ampule of glucose and admitted she ingested two pills of “street valium”
Patient recovered on HD 2; glucose level rebounded to normal.
Serum analysis revealed formula match for GLYBURIDE. Confirmed by retention time match to reference standard.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>$T_0$ 1198</td>
</tr>
<tr>
<td></td>
<td>$T_0 + 4.5$ hours 693</td>
</tr>
<tr>
<td></td>
<td>$T_0 + 8$ hours 590</td>
</tr>
<tr>
<td></td>
<td>$T_0 + 12.5$ hours undetectable</td>
</tr>
<tr>
<td>#2</td>
<td>$T_0$ 647</td>
</tr>
</tbody>
</table>

Therapeutic dose: 30-350 ng/mL
Toxic: > 600 ng/mL
Case

Two 17yo were brought in by ambulance after smoking Spike Maxx

Patient 1 - F, vomiting, slightly confused, T=36.3, HR=110, RR=20, K=3.1 mmol/L, no urine drug screen

Patient 2 - M, vomiting, very altered/confused, dilated pupils, cool skin, excess salivation, HR= 120, K= 3.0 mmol/L, Lactate= 4.4 mmol/L, NEGATIVE urine drug screen

Both patients were treated with antiemetics, IV fluid and were discharged 3, and 6hrs post-presentation
**SPICE**

- Most popular brand of HERBAL INCENSE

- Herbal incense - herbal blends originally intended as room deodorizers but are laced with synthetic cannabinoids

- Synthetic cannabinoids - class of compounds that mimic the effect of THC, the active component of cannabis

- THC binds to two types of receptors - CB1 and CB2

- SCs are originally synthesized to produce drugs with selective analgesic property

- Some SCs are more potent CB1 agonists

<table>
<thead>
<tr>
<th></th>
<th>CB1 $K_i$ (nM)</th>
<th>CB2 $K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THC</td>
<td>40.7</td>
<td>36.6</td>
</tr>
<tr>
<td>HU-210</td>
<td>0.234</td>
<td></td>
</tr>
<tr>
<td>CP 47,497</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>JWH-018</td>
<td>9</td>
<td>2.9</td>
</tr>
<tr>
<td>JWH-073</td>
<td>8.9</td>
<td>3.8</td>
</tr>
<tr>
<td>JWH-051</td>
<td>1.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Spice… A little History

1990’s Synthetic cannabinoids are synthesized in the lab of John W Huffman (JWH) at Clemson University
JWH-018 (1994)

2002 Spice first appeared in Asia as herbal incense
2004 Spice became available in Europe
2006 First incidences of Spice intoxication called in to Poison Control Centers in Europe
2008 Japan and Germany started analyzing herbal incense samples bought from headshops/ internet

Feb, 2009 First published articles on SPICE being adulterated with synthetic cannabinoids
Apr, 2009 German lab quantified adulterants in SPICE; more JWH compds found
April, 2010 JWH-018 metabolites identified
LEGAL STATUS OF SPICE IN THE US

Illegal

Kansas (02/2010) Louisiana ((07/18/2010)
Mississippi (04/2010) Hawaii (08/01/2010)
Kentucky (04/13/2010) Georgia (08/15/2010)

Temporary Ban/ Legislation Proposed

Arkansas New York
Florida Oklahoma
Iowa Utah
Maryland

Municipal Restriction

Indiana Texas
Minnesota Wisconsin

FDA Schedule I Drugs (March 1, 2011)
JWH-018, 073, 200, CP-47,497, Cannabicyclohexanol
Spike Maxx
Analysis suggests the presence of
  JWH – 007
  JWH – 073
  JWH – 398

Blood sample results
Both patients have 11-OH-THC
Girl’s serum has a formula match for JWH-018 (007 demethylates to 018)

We’ve developed pill, urine and serum synthetic cannabinoids assays.
## PCC Cases

**Cases Referred (2010): 55**  
**Cases Resolved: 43 (78%)**

<table>
<thead>
<tr>
<th>Case</th>
<th>No.</th>
<th>Drugs Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained Seizure</td>
<td>9</td>
<td>Venlafaxine, Tramadol, Diphenhydramine, Methadone, Dextromethorphan, Hydroxyzine,</td>
</tr>
<tr>
<td>Therapeutic Drug O/D</td>
<td>10</td>
<td>VPA, Atenolol, Sertraline, Trazodone, Ibuprofen, Retinoic acid, Propranolol, Verapamil,</td>
</tr>
<tr>
<td>Illicit Drug O/D</td>
<td>15</td>
<td>MDMA, Methadone, Propoxyphene, Clonazepam, Fentanyl, Dextomethorphan</td>
</tr>
<tr>
<td>Designer Drug</td>
<td>3</td>
<td>JWH compounds</td>
</tr>
<tr>
<td>Misrepresented Drug</td>
<td>4</td>
<td>Diazepam (Glyburide), Amphetamine (Zolpidem), Clomifene (Clenbuterol)</td>
</tr>
<tr>
<td>Adverse Drug Reaction</td>
<td>2</td>
<td>MDMA, acetaminophen</td>
</tr>
</tbody>
</table>
Non-Targeted Analysis of Environmental Toxins

New frontiers in expanding our understanding of chemicals in pregnant women (T Woodruff, A Zota, R Gerona)

**Hypothesis:** There are many more chemicals present in people’s bodies than currently identified through biomonitoring studies

**Specific Aims**
- To develop a comprehensive database of environmental chemical formulas
- To identify previously unmeasured candidate chemicals in the serum of pregnant women using unbiased interrogation methods
- To quantify the levels of 10-15 most commonly detected chemicals which have previously been unmeasured or underreported chemicals

**Approach:** Non-targeted analysis of environmental toxins in second trimester pregnant women using TOF LC-MS
Non-Targeted Analysis of Environmental Toxins

Sample Source and Study Population

- Serum samples from patients prior to second trimester pregnancy termination procedures at the San Francisco General Hospital Women’s Option Center (WOC)
- Patients are ethnically diverse and predominantly low income
  - 38% Black
  - 21% Hispanic
  - 21% Caucasian
  - 10% Asian
- Initially analyze stored serum samples for 20 pregnant women using a non-targeted approach for multiple environmental chemicals
- Analyze 30 prospectively collected serum samples using a targeted approach (qualitative and quantitative)
Non-Targeted Analysis of Environmental Toxins

Method
Sample Extraction: Protein precipitation/ SPE
Chromatography: C18 column with gradient elution (run time=8 min; re-equilibration= 2min)
Mass Spectrometry: TOF/MS using ESI positive, ESI negative, APCI positive, APCI negative (full scan between 100-1000 amu)
Output: Detected MWs, Mass accuracy, Target Score, Area under the curve
Criteria for Positive Hit: Mass accuracy ≤ 10ppm; Target Score ≥ 70; AUC ≥ 1000

Positive hits will be ranked according to detection frequency across 20 retrospective samples

Select top 10-15 positive hits for targeted quantitative analysis (LC-MS/MS) in 30 prospective samples
## Preliminary Results

Potential environmental toxins found in 3 patient serum samples by non-targeted analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Class</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Bisphenol S</td>
<td>Phenolic</td>
<td></td>
</tr>
<tr>
<td>BDE 47/66</td>
<td>PBDE</td>
<td>+</td>
</tr>
<tr>
<td>Monomethyl phthalate</td>
<td>Phthalate</td>
<td>+</td>
</tr>
<tr>
<td>Monoisononyl phthalate</td>
<td>Phthalate</td>
<td>+</td>
</tr>
<tr>
<td>Anabasine</td>
<td>Insecticide</td>
<td>+</td>
</tr>
<tr>
<td>DEET</td>
<td>Insecticide</td>
<td>+</td>
</tr>
<tr>
<td>DNOC</td>
<td>Insecticide</td>
<td>+</td>
</tr>
<tr>
<td>Ethoxyquin</td>
<td>Herbicide</td>
<td>+</td>
</tr>
<tr>
<td>Malathion</td>
<td>Insecticide</td>
<td>+</td>
</tr>
<tr>
<td>Zectran</td>
<td>Insecticide</td>
<td>+</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Herbicide</td>
<td>+</td>
</tr>
</tbody>
</table>
Acknowledgement

Dr. Alan Wu

Dr. Kent Olson, Dr. Tom Kearney, Dr. Paul Blanc, Dr. Craig Smollin, Dr. Patil Armenian, Dr. Derrick Lung, Dr. Elisabeth Birdsall

Dr. Tracey Woddruff, Dr. Ami Zota, Carrie

Dr. Deborah French, Jean Branch, Dr. Kathy Chen

Dr. Richard Ko
Additional Slides
Herbal X

- Claims to increase libido
- Most ingredients have been studied for their role in supporting healthy sexual performance and increased libido
- Listed ingredients: Acorus rhizome, Alpinia fruit, Black walnut seed, Cherokee Rose fruit, Cimicifuga rhizome, Cordyceps mycelium, Cornus fruit, Glycyrhiza root, Lycium fruit, Panax Ginseng root, Poria sclerotium, Rehmania root

MS-TOF Qualitative Analysis

- Methanol extract of Herbal X contains very high formula matches for
  - acetildenafil (structural analogue of Viagra)
  - hydroxyhomosildenafil (derivative of Levitra)
- Both compounds have been reported as dopants used in herbal supplements for erectile dysfunction.
### Analysis of Herbal Supplements

<table>
<thead>
<tr>
<th>Name</th>
<th>Score (MFG)</th>
<th>RT</th>
<th>Mass</th>
<th>Diff (MFG, ppm)</th>
<th>Formula (MFG)</th>
<th>Ions</th>
<th>Height</th>
<th>Area</th>
<th>Abund</th>
<th>File</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetildenafil</td>
<td>84.05</td>
<td>4.147</td>
<td>466.27</td>
<td>-1.7</td>
<td>C25 H34 N6 O3</td>
<td>4</td>
<td>1548390</td>
<td>4476039</td>
<td>483578</td>
<td>HerbalX-1.d</td>
</tr>
<tr>
<td>Hydroxyhomosildenafil</td>
<td>91.08</td>
<td>4.602</td>
<td>488.2208</td>
<td>-0.53</td>
<td>C23 H32 N6 O4 S</td>
<td>7</td>
<td>1118384</td>
<td>2318706</td>
<td>298117</td>
<td>HerbalX-1.d</td>
</tr>
</tbody>
</table>

**Chemical formulas:**

- Sildenafil: $R=H$
- Homosildenafil: $R=\text{CH}_3$
- Hydroxyhomosildenafil: $R=\text{CH}_2\text{OH}$
- Acetildenafil

**Diagram:**

- Sildenafil
- Homosildenafil
- Hydroxyhomosildenafil
Case
54 yo M with complex medical history had a suicide attempt. In the ER, his mental status precipitously diminished leading to intubation with need for sedation/ paralysis.
Patient had a heart attack; while being resuscitated, patient developed **Torsades de Pointes** which resolved after CPR, IV MG, and IV NaHCO₃. EKG showed a prolonged QT >600msec

List of Medications

- Aspirin
- Atorvastatin
- Buspirone
- Fluoxetine
- Levothyroxine
- Metformin
- Metoprolol
- Trazodone

Which medication did he overdose on that may have caused torsades?
## Solving a puzzle

### Drugs with formula and RT matches in patient’s serum:

- Aspirin, Fluoxetine, Metformin, Metoprolol, Trazodone

### Drugs not detected in patient’s serum:

- Atorvastatin, Buspirone, Levothyroxine

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trazodone (mcg/mL)</th>
<th>Metformin (ng/mL)</th>
<th>Fluoxetine (ng/mL)</th>
<th>Norfluoxetine (ng/mL)</th>
<th>Metoprolol (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02/08 1215H</td>
<td>4.06</td>
<td>159.78</td>
<td>20.11</td>
<td>11.0</td>
<td>10.9</td>
</tr>
<tr>
<td>02/08 2120H</td>
<td>4.09</td>
<td>101.69</td>
<td>34.07</td>
<td>23.7</td>
<td>7.1</td>
</tr>
<tr>
<td>02/10 0420H</td>
<td>2.74</td>
<td>16.97</td>
<td>39.86</td>
<td>31.4</td>
<td>15.5</td>
</tr>
<tr>
<td>02/11 0505H</td>
<td>2.47</td>
<td>9.41</td>
<td>40.97</td>
<td>32.5</td>
<td>7.7</td>
</tr>
<tr>
<td>02/16 0445H</td>
<td>0.0276</td>
<td>4.76</td>
<td>22.50</td>
<td>13.8</td>
<td>8.9</td>
</tr>
<tr>
<td>Therapeutic level</td>
<td>0.5-2.5</td>
<td>1000-4000</td>
<td>150-500</td>
<td></td>
<td>100-600</td>
</tr>
<tr>
<td>Toxic level</td>
<td>4.0</td>
<td>5000</td>
<td>&gt;1300</td>
<td></td>
<td>650</td>
</tr>
</tbody>
</table>
Studying the Progress of VPA Intoxication

CASE
22 yo F O/D with valproic acid
Comatose
Hypernatremic, hypocalcimic,
initial AG= 18

AIM
Monitor metabolites relevant in VPA toxicity

USE of MS-TOF
Simultaneous analysis of metabolites in patient serum samples drawn intermittently at the ICU

Carnitine given
Studying Progress of VPA Intoxication

- Valproic acid uses up carnitine leading to carnitine deficiency
- Cells cannot transport long chain fatty acids into the mitochondrion for their metabolism through β-oxidation
- Cells shift to ω-oxidation to metabolize long chain fatty acids- increase in adipic and suberic acids, metabolites of ω-oxidation
- Metabolism of valproic acid leads to production of its toxic metabolites, 2/4-en-VPA
VPA Intoxication

- **Valproic acid**
  - Carnitine given
  - VPA normalized

- **Adipic acid**
- **Suberic acid**

- **Acetylcarnitine**

- **VP-carnitine**
- **2en/4en-VPA**

**Time after Admission (hr)**

**Peak Area (AU)**
A Case of Mistaken Identity

Case

Patient presented to the ED after taking 0.5mL Clomifene citrate (obtained from the black market) sublingually. Patient had chest discomfort, palpitations, tremor and anxiety. Patient claimed that he felt his heart rate suddenly racing 5 minutes after taking the drug. EKG confirmed narrow complex tachycardia with the rate of 150.

Patient was taking testosterone obtained illicitly for body building. He self-prescribed Clomifene to counteract the feminizing effects of testosterone intake.

A sample of Clomifene purchased by the patient was sent to SFGH for analysis. Attending was suspicious that the bottle actually contains clenbuterol.

Sample Analysis

Sample contains CLENBUTEROL at 28.45mg/mL. NO CLOMIFENE was detected.

Recommended dosage: <120mcg/day

Patient Intake with 0.5mL = 14.2mg
Chemical Exposures During the Second Trimester of Pregnancy

(Project in collaboration with Dr. Tracey Woodruff)

Specific Aims:
1. To describe the fetal exposure to BPA during the second trimester and explore the feasibility and accuracy of using maternal biological monitoring results to predict fetal exposure
2. To investigate predictors of maternal and fetal exposures to BPA during second trimester of pregnancy
3. To characterize hepatic metabolism of BPA in the fetal and adult liver

Study will recruit 100 women undergoing elective pregnancy terminations between 14 and 23 weeks gestation.

Develop method to measure BPA, BPA glucuronide and BPA sulfate in maternal urine, maternal serum, umbilical cord blood, placenta and amniotic fluid