CHALLENGES IN FORENSIC TOXICOLOGY

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Objectives

- **Why am I here**
  - Give a quick overview of how we approach casework in postmortem toxicology
  - Discuss analytical and legal challenges we face on a routine basis and get participant feedback

- **What I hope to accomplish today**
  - Get you an understanding of what it is we do, general approach
  - Clearly express some of the problems we face
  - Promote some feedback or discussion on how we may improve the field and the practice.
Outline

• Overview of Postmortem Forensic Toxicology
• Current Analytical Approaches
• Case example
• Challenges
  • Interpretive
  • Legal
  • Analytical
  • Industry
• Research / Development
• Discussion
What is Forensic Toxicology?

Toxicology

The study of the adverse effects of chemicals on living organisms

Forensic Toxicology

The science of the detection and interpretation of drugs and poisons in the human body for the purpose of medicolegal investigations.
Applications of Forensic Toxicology

- Human Performance Toxicology
  - DUI/DWI
    - Blood/Urine Drugs and Alcohol Testing
    - Breath Testing Programs
    - Drug Facilitated Sexual Assault
- Workplace Drug Testing
- Postmortem Toxicology
Postmortem Toxicology

- Answer this Question: Did drug or alcohol use cause or contribute to a person’s death?
  - Determine the absence or presence (and concentration) of drugs and chemicals in human biological specimens
  - Helps medical examiners in the determination of cause and manner of death
  - Produces legally defensible results
Recent Overdose Deaths

- Correy Monteith (Actor, Musician), Philip Seymour Hoffman (Actor)
  - Heroin, Alcohol (Accident)
- Anna Nicole Smith, Model
  - Combined Drug intoxication: sleeping medication chloral hydrate as the "major component." (in combination with other prescription drugs) (Accident)
- Michael Jackson, Musician
  - Acute propofol intoxication (Homicide)
- Amy Winehouse, Musician
  - Acute Alcohol Poisoning
- Derek Boogaard, Hockey Player - NY Rangers
  - Drug overdose: Oxycodone and alcohol (Accident)
- Heath Ledger, Actor
  - Toxic combination of prescription drugs: Oxycodone, Hydrocodone, Diazepam, Alprazolam, Doxylamine (Accident)
Medicolegal Death Investigation

Scene Investigation

Autopsy

Toxicology
Analytical - Process

• Forensic testing is highly regulated with multiple safeguards built into the system to protect the rights of individuals tested.
• Quality of result is only as good as the quality of the entire process.
Analytical - Process

- Safeguards begins at collection site (Autopsy)
- Transportation of specimen to testing laboratory
- Accuracy of accessioning the specimens
- Testing
- Data Review / Generation of Report
- Conservative interpretation of results
Analytical - Process

• **Forensic Toxicologist**
  • Accessioning, Screening, Extractions
    • Producing quality results in a timely manner

• **Senior Forensic Toxicologist**
  • Data Review, Method Development, Troubleshooting, Quality Assurance and Quality Control oversight

• **Deputy Chief / Chief Toxicologist**
  • Data Review, Case Sign-out (release of result), Managerial duties, Oversee the entire Laboratory
Analytical - Process

• A single Forensic Toxicologist
  • Handles the entire case from start to finish

• Issues with turn around time

• Issues with training and acquired skill set
Analytical – Targeted Analytes

- Gases
- Volatile Substances
- Miscellaneous Substances
- Drugs
- Pesticides
- Metals
- Anions

Analyte
Analytical - Strategy

Toxicology PROTOCOL

- Homicide
  - Volatiles EIA Screen
- General Unknown
  - Volatiles EIA Screen
  - Basic Drug Screen
- Infants - Elderly
  - Volatiles EIA Screen
  - Basic Drug Screen
  - Acid Neutral Screen
  - LC/MS/MS MRM Screen
- Automobile Accident
  - Volatiles EIA Screen
  - Basic Drug Screen
Analytical - Extractions

• None (Volatile, ELISA)

• Liquid-Liquid

• ACN Crash

• Solid Phase Extraction (SPE) – mixed mode 200mg bed
Analytical – Instrumental

• ELISA

• Colormetric Tests, UV

• GC/MS/NPD

• HS/GC – FID

• LC/MS/MS

• QTOF (Recent)
Putting it together
Case Study – Taken from Literature

- Teenage male found unresponsive and transported to hospital while receiving CPR
- Medical history of pulmonary issues, pneumonia, sleep apnea, “severe respiratory compromise”
- No known ethanol or illicit drug history.
- Investigations reported that he “sometimes took a pill to energize self”.

Case Study – Approach

- Analysis for volatile compounds
- Analysis by ELISA for drugs of abuse
- Analysis by GC/MS/NPD for drugs of abuse and typical chemically basic medications
- Analysis by LC/MS for NSAID compounds
Case Study – Approach

- ELISA is positive for benzodiazepines
- Base screen is positive for diazepam, nordiazepam, and a significant unidentified peak
Case Study – Base Screen (TIC)
Case Study – Mass Spectrum

- Literature Review
- Discussed mass spectrum with colleagues
Case Study – Unknown Drug: Tapentadol
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Case Study – Unknown Drug: Tapentadol
Case Study – Unknown Drug: Tapentadol
<table>
<thead>
<tr>
<th>Source</th>
<th>Tapentadol (mg/L)</th>
<th>N-Desmethyl Tapentadol</th>
<th>Diazepam</th>
<th>Nordiazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral Blood</td>
<td>0.77</td>
<td>0.07</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Heart Blood</td>
<td>1.95</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.24</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>1.65</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine (Free)</td>
<td>1.60</td>
<td>None Detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine (Total)</td>
<td>0.74</td>
<td>.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Case Study – Tapentadol

- Cause of death: “Mixed Drug Toxicity” with contributory condition “Obesity; Dilated Cardiomyopathy”
- Manner of death: “Accident”
- The tapentadol blood ranges were in range of previously reported deaths
- This is the first tissue distribution of tapentadol and N-desmethyltapentadol
- Tapentadol is used to treat moderate to high levels of non-specific pain
Challenges

INTERPRETATION
Challenges – Interpretation

Pharmacology / Physiology
Tolerance
Drug Stability
Redistribution
Challenges – Interpretation

• **Drug Metabolites**
  - Metabolites may be active and can add to toxicity
  - Ratio of parent to metabolite $\rightarrow$ chronic vs. acute
  - Individual variations in metabolism

• **Drug Interactions**
  - Drugs of the same class are often additive in effects
  - Drugs with opposing effects $\rightarrow$ various effects
Challenges – Interpretation

Tolerance

• Accommodation to the effects of the drug
• Will require increasing dose to achieve same effect
• Concentration ranges will overlap (terminally ill & incarceration)
• Problem with naive opiate users
Challenges – Interpretation

Postmortem Redistribution

• Drug dependant factors:
  • Molecular size
  • Lipophilicity
  • pKa
  • Energy-dependant transport
  • Tissue affinity for the drug
Challenges – Interpretation

Postmortem Distribution

Volume of Distribution (L/kg)
Challenges – Interpretation

- Diffusion of drug from high concentration to a lower concentration
  - muscle to blood vessel
  - Fat to blood vessel
Challenges – Interpretation

- Diffusion of drug from high concentration to a lower concentration
  - muscle to blood vessel
  - Fat to blood vessel

Hours later

10 mg/L
20 mg/L
500 mg/L
## Challenges – Interpretation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Volume of Distribution (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.8 – 1.0</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>6 – 10</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>3.2 – 5.6</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.6 – 2.7</td>
</tr>
<tr>
<td>Doxepin</td>
<td>9-33</td>
</tr>
<tr>
<td>Diphendydramine</td>
<td>3 – 14</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.43 – 0.59</td>
</tr>
<tr>
<td>Imipramine (TCA)</td>
<td>20 – 40</td>
</tr>
<tr>
<td>Methadone</td>
<td>4 – 5</td>
</tr>
<tr>
<td>Morphine</td>
<td>2 – 5</td>
</tr>
</tbody>
</table>
Challenges

LEGAL / ANALYTICAL
Challenges - Legal

- **Daubert Standard**
  - Rule of evidence regarding the admissibility of expert witness's testimony during United States federal legal proceedings.
  - Empirical testing: the theory or technique must be falsifiable, refutable, and testable (Uncertainty).
  - Degree to which the theory and technique is generally accepted by a relevant scientific community (Subjected to peer review/publication).
  - The existence and maintenance of standards and controls concerning its operation (QA/QC).
Challenges - Regulatory

• REGULATIONS
  • Institutional (ABFT/ASCLD)
  • Local (DUI legislation)
  • Federal (SAMSHA)
Challenges - Spectrophotometry Technologies

- CO, NSAID, Cardiac medications
- LC/Diode Array not applied as often due to the development of LC/MS
- Limited due to interference and sensitivity
- 2D-LC could revitalize this in modern laboratories
Challenges - GC/MS Technologies

- OLDER but inexpensive, highly dependable, and defensible
- DOT Drug Testing
- Lengthy extractions
- Derivitization (stability, chromatography)
- Cost of helium
Challenges - MS/MS Technologies

• TANDEM MS
• Cost of ACN
• Specific Transitions are good for specific screening
• Transitions can vary depending on instrumentation
• Sensitivity can lead to carryover issues
• “Crosstalk” transition issues (Cathinones)
  • Solve with retention time, issues with speed of analysis
Challenges - Accurate Mass Technologies

• TOF / MS
• Much better for non-specific analysis
• Huge amount of data collection
• Limited, custom built libraries – needs standardization (for fragments)
• Limited by extraction
Challenges – Typical Specimens

Peripheral Blood
Central Blood

Urine
Vitreous Humor
Bile

Liver
Brain
Gastric Contents

Hospital Admission Bloods
Challenges – Atypical Specimens

Kidney
Spleen

Syringes
Pipes
Household Products

Hair
Nails
Insects
Challenges - Matrix Availability

- Substitute Matrixes
- Commercial Matrixes
- Available Matrix – Unexpected Positives
Challenges - Analytical Standards

- Availability
- Traceability
- Laboratory Requirements (ABFT, ASCLD)
- Synthesis
Challenges – Novel Psychoactive Substances

- **Cathinones**
  - MDPV
  - Methylone
  - Mephedrone (4-MMC)
  - Fluoromethcathinone
  - 4-MEC
  - Alpha-PVP

- **Synthetic Cannabinoids**
  - JHW-250
  - AM-2201
  - JHW-122
  - PB-22
  - 5F-PB-22
  - 5-Chloro-UR-144
  - A-334,735
  - UR-144 N-Heptyl homolog
Challenges – Novel Commercial Compounds

• Antipsychotics
• Antidepressants
• Oncology
• Sleep Disorders
• Musculoskeletal, Trauma (pregabalin)

• Compounded products from pharmaceutical labs
Challenges - Industry

• Forensics is not a money making business

• Doesn’t attract vendors, chemists, researchers, universities...
Future Screening Techniques

- Traditional Techniques vs New Applications
- TOF
- Aptamers vs. ELISA?
Future Research

• Traditional Techniques vs New Applications

• TOF? TOF Libraries?

• Aptamers vs. ELISA?

• Biomarkers (Postmortem) and impurities

• 2D-Chromatography
Discussion