Analysis of Biological Specimens for the Presence of Novel Psychoactive Substances from Attendees at an Electronic Dance Music Festival

Mandi Mohr, M.S.
Disclaimer

The project was supported by Award No. 2013-DN-BX-K018, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.
Novel Psychoactive Substances

• Emergence of “designer drugs,” “legal highs,” and/or “club drugs” has seen a steady increase since early 2008

• NPS refers to a broad category of products containing unregulated phenethylamines, cathinones, and synthetic cannabinoids

• Market is in constant flux
  – Limited ability to develop analytical methods in a timely fashion
  – Limited research on metabolism
Electronic Dance Music Festivals

• U.S. Electronic Dance Music (EDM) is characterized by techno-rock music and rave culture

• Literature sources indicate as many as 70% of attendees may be using recreational drugs

• Emphasis on harm reduction, public health and awareness
  – Dancesafe.org
  – Bunk Police
NPS at EDM Festivals in the U.S.

• Ultra Music Festival – Miami, Florida
  – 2013: 1 death
  – COD: Acute Methylone Toxicity – 1.2 mg/L

• Electric Zoo Festival – New York, New York
  – 2013: 2 deaths
  – COD: Acute MDMA intoxication with hyperthermia

• Bonnaroo Music Festival – Manchester, TN
  – 2013: 2 deaths
  – COD: Information unavailable
Project Design and Objectives

• Collection and analysis of paired blood, urine, and oral fluid samples

• Project goals included:
  – Identify novel drugs on the market
  – Determine prevalence of designer drugs
  – Characterize potential metabolites using in vitro studies and authentic samples
SAMPLE COLLECTION
Location

- Approximately 100 yards from entrance gate
- Able to obtain volunteers from heavy foot traffic
  - Location between entrance and public transportation stop
Sample Collection

• Step 1: Consent forms/Survey

• Step 2: Oral Fluid Collection
  – Immnualysis Quantisal™
  – Alere™ DDS2® Mobile System

• Step 3: Urine

• Step 4: Blood

Disclosure: Participants were not required to donate all 4 samples, and only donated samples based on their comfort level. The gift card incentive was only given if the participant donated a blood sample.
Sample Set

- Collected samples from 145 subjects
  - 105 urine samples
  - 65 blood samples
  - 125 oral fluid samples screened with the Alere™ DDS2® Mobile System
  - 136 oral fluid samples collected with the Immunalysis Quantisal™
DEMOGRAPHICS AND SURVEY RESULTS
Demographics

• Samples were collected from 76 males and 60 females
  – 9 subjects did not indicate M/F

• Average age: 23.7 (±6.4)
  – Range: 18-57 years old

• 72% of participants indicated they had taken a recreational or medicinal substance in the last week
  – THC was most common answer, followed by Cocaine
Responses of Drug Use

- THC: 57%
- Cocaine: 16%
- MDMA: 14%
- Molly: 14%
- Ecstasy: 13%
- Alcohol: 13%

n=104
Self-Reported NPS Use

• 30 participants reported taking MDMA, “Molly” or “Ecstasy” within the last week

• Typically routes of administration were orally via a pill or capsule

• Self-reported dosages:
  – MDMA: 0.3 grams up to 25 grams
  – Molly: 0.2 mg up to 2.3 grams
ORAL FLUID ANALYSIS AND RESULTS
Oral Fluid On-Site Screening

- Oral fluid samples were screened on-site after participants had left the study area.
- Samples were screened with the Alere™ DDS2® Mobile System.
- Device screens for common drugs of abuse.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Alere DDS2 Cutoffs (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>50 (Amp)</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>20 (Temazepam)</td>
</tr>
<tr>
<td>Cannabis</td>
<td>25 (THC)</td>
</tr>
<tr>
<td>Cocaine</td>
<td>30 (BZE)</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>50 (mAmp)</td>
</tr>
<tr>
<td>Opiates</td>
<td>40 (Morphine)</td>
</tr>
</tbody>
</table>
Oral Fluid Screening Results

- 125 oral fluid samples tested using the Alere DDS2
- Confirmatory results pending

<table>
<thead>
<tr>
<th></th>
<th>Cannabis</th>
<th>Cocaine</th>
<th>Opiates</th>
<th>Meth</th>
<th>Amp</th>
<th>Benzos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27</td>
<td>12</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>92</td>
<td>107</td>
<td>119</td>
<td>117</td>
<td>118</td>
<td>120</td>
</tr>
<tr>
<td>Invalid</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>% Positive</td>
<td>22.13%</td>
<td>9.84%</td>
<td>0%</td>
<td>2.46%</td>
<td>2.46%</td>
<td>0.82%</td>
</tr>
</tbody>
</table>
BLOOD SAMPLE ANALYSIS & RESULTS
Sample Analysis

- Samples were screened on a Waters ACQUITY UPLC® I Class Waters Xevo® G2-S QTOF

- Confirmations were either via 2D-GC/MS or LC-MS/MS
  - Common drugs of abuse confirmed at NMS Labs
Blood Screening Results

• 73% (48 subjects) of the blood samples screened positive for a common drug of abuse/metabolite or NPS substance
  – Most common drug of abuse: THC followed by cocaine

• Of the 48 subjects screening positive, 41% had at least one NPS or MDMA detected that were sent for confirmatory testing
% Positive Rate in Blood Samples

- THC: Very high positive rate
- Alcohol: High positive rate
- NPS: Moderate positive rate
- Amines: Low positive rate
- Cocaine: Moderate positive rate
- Benzodiazepines: Low positive rate
- Opiates: Very low positive rate
### NPS Analytes

<table>
<thead>
<tr>
<th>NPS Analyte</th>
<th>4-FA</th>
<th>Alpha-PVP</th>
<th>Dimethylone</th>
<th>Methylone</th>
<th>MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Positive Samples (n=21)</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

- NPS analytes detected were commonly discussed stimulants on EDM forum websites
- Typical effects of these analytes include:
  - Euphoria, alertness, sexual arousal, focused mind, and overall positive feeling
“Molly”

- Several participants indicated they had taken “Molly” within the last week
- Samples of subjects who reported taking “Molly” contained:
  - MDMA
  - Alpha-PVP
- Samples of subjects who reported taking MDMA contained:
  - Methylone
  - Dimethylone
  - Alpha-PVP
URINE SAMPLE ANALYSIS & RESULTS
Urine Sample Analysis

• Urine sample analysis occurred at the Armed Forces Medical Examiner System Division of Forensic Toxicology

• Samples were screened via several analytical techniques
  – Immunoassay, GC-MS, LC-QTOF, and RapidFire tandem mass spectrometry

• Samples were confirmed using either GC/MS or LC-MS/MS
Summary of Urine Analysis

- Negative: 35%
- Alcohol Only: 8%
- Single Drug Positive: 21%
- 1 Drug + Alcohol: 5%
- Multiple Drug Positive: 16%
- Multiple Drugs + Alcohol: 15%
% Positive Rate in Urine Samples

- THC: 50%
- NPS: 30%
- Alcohol: 25%
- Cocaine: 15%
- Basic Drugs: 10%
- Amines: 5%
- Benzodiazepines: 2%
- Opiates: 1%
IN VITRO METABOLITE DISCOVERY
Human Liver Microsome Incubations

• Samples were prepared by adding:
  – 100 mM Phosphate Buffer (pH 7.4) with 10 mM MgCl₂
  – 5 µL of drug of interest (1 mg/mL)
  – 50 µL of 10 mM NADPH
  – 25 µL of pooled human liver microsomes

• Samples were incubated in a water bath for 2 hours at 37⁰C

• Samples were analyzed on a Waters ACQUITY UPLC® I Class Waters Xevo® G2-S QTOF
NPS Analytes of Interest

• Alpha-PVP
  – Belongs to the “bath salts” group of substances
  – Published literature has examined the metabolites using HLM, rat urine, and human urine

• Methylone and Dimethylone
  – Isomers
  – Published literature on methylone metabolism
  – Hypothesized that dimethylone will metabolize into methylone and follow similar metabolic pathway
Alpha-PVP HLM Incubations
Alpha-PVP Metabolism
Alpha-PVP Metabolites in Blood

The bar chart shows the levels of various metabolites in blood, with Alpha-PVP (Major) and 2'-oxo-PVP being the most prominent. Other metabolites include 5-OH-PVP (Minor), Butylamino, OH-Alkyl-PVP, OH-Alkyl-PVP, OH-Phenyl-PVP 2, OH-Phenyl-PVP 1, Carboxyamino-PVP, and Amino-PVP.
Alpha-PVP Metabolites in Urine

![Graph showing the levels of Alpha-PVP and its metabolites in urine. The x-axis represents different metabolites, and the y-axis represents the concentration levels. The metabolites are listed as follows: Alpha-PVP, 5-OH-PVP, Butylamino, OH-Alkyl-PVP, 2'-oxo-PVP, OH-Alkyl-PVP, OH-Phenyl-PVP 2, OH-Phenyl-PVP 1, Carboxyamino-PVP, Amino-PVP. The concentrations range from 0 to 14. The graph indicates that 5-OH-PVP and Butylamino, OH-Alkyl-PVP have the highest concentrations.]
Dimethylone Metabolism

Dimethylone

Demethylation

Methylation

Methylone

Demethylation

Demethylation
Discussion

• Collaborative efforts spanning private, government, research, and academic institutions led to expedited sample analysis

• Blood, urine, and oral fluid samples collected demonstrated high levels of NPS use among EDM festival attendees
  – Many participants are unknowingly ingesting highly potent substances with increased adverse side effects raising a public health concern
Conclusion

• Identify novel drugs on the market – unknown chromatographic peaks were detected and are being investigated

• Determine prevalence of designer drugs – 30% of participants were confirmed positive for a NPS in either the blood or urine
  – Majority of subjects who are drug positive are poly drug cases
Conclusions

• Characterize potential metabolites using in vitro studies and authentic samples - Alpha-PVP metabolites are seen both in blood and urine samples
  – 5-OH and 2’’-oxo metabolites are most commonly seen in blood
    • At higher concentrations of the parent compound, other metabolites may be seen
  – All major metabolites produced with the HLM incubations are seen in the urine
Future Work

• Returning to the EDM festival in 2015 to collect additional samples
  – Monitor trends in NPS use
  – Add additional collection sites

• Continued collaboration with AFMES

• Continue work with HLM incubations
  – Development of a metabolite database to implement in testing

• Future to collaborations with Bellevue Hospital and Jackson Memorial Hospital to analyze urine samples collected from the general population suspected of using NPS
  – Reconcile findings between EDM population and general population
Acknowledgements

• The National Institute of Justice
• Waters Corporation
• The Armed Forces Medical Examiners System Division of Forensic Toxicology
• Immunalysis Corporation
• NMS Labs
• RMI Laboratories
• Arcadia University
• Dr. Barry Logan, Melissa Friscia, Jill Yeakel
• Fran Diamond, Sarah Wolf, Helen Piper
The Research Team
Questions?

mmohr@frfoundation.org