New drugs of abuse?
A case study on
Phenazepam & Methoxetamine

Presenter: Nadia Wong
Co-authors: Dr Yao Yi Ju & Alex Low Xuan Kai

Analytical Toxicology Laboratory
Clinical & Forensic Toxicology Unit
Applied Sciences Group
Health Sciences Authority
Singapore

Forensic & Clinical Toxicology Association
31 July - 3 August 2011, Melbourne, Australia
Content

- Aim
- Intro to Phenazepam
- Intro to Methoxetamine
- Case History
- Specimens Analysis
- Conclusion
Aim

• To present a case on potential abuse of Phenazepam and Methoxetamine in Singapore.
# Intro to Phenazepam

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IUPAC name</strong></td>
<td>[7-bromo-5(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one]</td>
</tr>
<tr>
<td><strong>Molecular Weight</strong></td>
<td>349.61</td>
</tr>
<tr>
<td><strong>Molecular Formula</strong></td>
<td>C_{15}H_{10}BrClN_{2}O</td>
</tr>
<tr>
<td><strong>Half-life</strong></td>
<td>About 60 hrs</td>
</tr>
</tbody>
</table>

## History
Drug is currently in clinical use in Russia as a sedative-hypnotic drug since 1978. Dosage is usually administered at 0.5mg 2-3 times daily.

## Effect Classification
Tranquilizer (class of benzodiazepine)

## Metabolism
![Hydroxylation](image)

## Drug effect
Dizziness, incoordination, asthenia and somnolence

Information are obtained from "Disposition of Toxic Drugs and Chemicals in Man", 8th ed, R.C. Baselt, Biomedical Publications, Foster City, California (2008)
# Intro to Methoxetamine (MXE)

<table>
<thead>
<tr>
<th>IUPAC name</th>
<th>2-(3-methoxyphenyl)-2-(ethylamine) cyclohexanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>247.33</td>
</tr>
<tr>
<td>Molecular Formula</td>
<td>C$<em>{15}$H$</em>{21}$NO$_2$</td>
</tr>
<tr>
<td>Half-life</td>
<td>Not known</td>
</tr>
</tbody>
</table>

**History**

Currently no reported medicinal use. First publicly reported in 2010. A ketamine alternative for the laboratory research.

**Effect Classification**

Sedative, dissociative anesthesia

**Metabolism**

Not known

**Drug effect**

Hallucination and euphoria
Intro to Methoxetamine

- Arylcyclohexylamine class of chemical
- Analogue of ketamine
- Rational drug design

![Chemical structures](image)
Intro to Methoxetamine

- Arylcyclohexylamine class of chemical
- Analogue of ketamine
- Rational drug design

Controlled Drug in Singapore

Analogue

Ketamine

Methoxetamine
# Appearance of Drugs

<table>
<thead>
<tr>
<th>Phenazepam</th>
<th>Methoxetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Phenazepam" /></td>
<td><img src="image2.png" alt="Methoxetamine" /></td>
</tr>
<tr>
<td>White crystalline powder</td>
<td>White crystalline powder</td>
</tr>
</tbody>
</table>

Potential Drugs Abuse?

- Can be readily purchased through internet
- Information of drugs available on internet
  - Method and dosage of consumption
  - Sharing of experience by drug users
- Not controlled under the law
  - Singapore
  - USA
  - UK
Case History

1st case: 3. Apr. 2011
- 39 years old Chinese man with HIV positive
- Found unconscious in hotel room
- Symptom: slowness in motion
- Found 2 medications: Phenazepam & 2-(3-methoxyphenyl)-2-(ethylamine)cyclohexanone

2nd case: 3. Apr. 2011
- 43 years old Caucasian man
- Found unconscious on floor
- Symptom: Drowsiness
- Found 2 medications: Phenazepam & 2-(3-methoxyphenyl)-2-(ethylamine)cyclohexanone
Clinical Case

• Specimens were sent to Analytical Toxicology Laboratory for drugs screening.

• Specimens Type:
  ➢ Whole Blood (heparinised) 3 ml
  ➢ Urine 20 ml
Specimens Analysis

Blood/Urine
Liquid-liquid Extraction
GC/MS
LC/TOF-MS
Specimens Analysis

- Blood/Urine
- Liquid-liquid Extraction
- GC/MS
- LC/TOF-MS
Basic Drugs Extraction (LLE)

1 ml blood/urine

0.5 ml basic carbonate buffer (pH 12 ca.), vortex

5 ml 1-Chlorobutane containing Diphenoxylate (I.S), shake, centrifuge

4 ml supernatant, 100 μl acidic MeOH, evaporate under N₂

Reconstitute 100 μl MeOH

Instrument
Specimens Analysis

Blood/Urine

Liquid-liquid Extraction

GC/MS

LC/TOF-MS
Instrumentation: GC/MS

Agilent 7890A/5975 inert GCMS

**GC condition:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>HP-5MS(25 m x 0.20 mm i.d. x 0.33 µm film thickness)</td>
</tr>
<tr>
<td><strong>Oven equilibration time</strong></td>
<td>0.50 min</td>
</tr>
<tr>
<td><strong>Initial temperature</strong></td>
<td>100 °C hold 1 min</td>
</tr>
<tr>
<td><strong>Temperature programming</strong></td>
<td>10 °C/min to 300 °C (hold 12 min)</td>
</tr>
<tr>
<td><strong>Total run time</strong></td>
<td>33 min</td>
</tr>
<tr>
<td><strong>Injection temperature</strong></td>
<td>300 °C</td>
</tr>
<tr>
<td><strong>Purge time</strong></td>
<td>0.3 min</td>
</tr>
<tr>
<td><strong>Injection volume</strong></td>
<td>2 µl</td>
</tr>
<tr>
<td><strong>Injection mode</strong></td>
<td>Splitless</td>
</tr>
<tr>
<td><strong>Flow (constant)</strong></td>
<td>1 ml/min (He)</td>
</tr>
</tbody>
</table>
Instrumentation: GC/MS

Agilent 7890A/5975 inert GCMS

MS condition:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition mode</td>
<td>El scan</td>
</tr>
<tr>
<td>Mass range</td>
<td>50 to 500 (m/z)</td>
</tr>
<tr>
<td>Threshold</td>
<td>150</td>
</tr>
<tr>
<td>MS transfer line temperature</td>
<td>280 °C</td>
</tr>
<tr>
<td>Source temperature</td>
<td>230 °C</td>
</tr>
<tr>
<td>Quadrupole temperature</td>
<td>150 °C</td>
</tr>
<tr>
<td>Solvent delay</td>
<td>1.5 min</td>
</tr>
</tbody>
</table>
GC/MS Chromatogram of a blood extract
GC/MS Chromatogram of a urine extract

I.S DIPHENOXYLATE

Unknown

Methoxetamine?
GC/MS Spectrum
Unknown samples vs White crystal found

Quality match = 97%

Unknown peak

White crystal
Library from Prof. Dr Hans H. Maurer

entry number 8506 in 2011 Maurer/Pfleger/Weber library
Specimens Analysis

- Blood/Urine
- Liquid-liquid Extraction
- GC/MS
- LC/TOF-MS
Instrumentation: LC/TOF-MS

Agilent HP1100 HPLC series

Chromatographic condition:

<table>
<thead>
<tr>
<th>Column</th>
<th>150 x 2.1 mm, Hypersil-BDS column (5 µm particle size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase A</td>
<td>2 mM ammonium formate &amp; 0.2% formic acid in water</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>2 mM ammonium formate &amp; 0.2% formic acid in methanol</td>
</tr>
<tr>
<td>Column temp.</td>
<td>25 °C</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.4 ml/min</td>
</tr>
<tr>
<td>Volume injection</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Gradient elution programme:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>26</td>
<td>80</td>
</tr>
<tr>
<td>26.1</td>
<td>5</td>
</tr>
<tr>
<td>32</td>
<td>5</td>
</tr>
</tbody>
</table>
Instrumentation: LC/TOF-MS

Agilent 6210 Times-of-Flight Mass Spectrometer

MS TOF condition:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ion polarity</strong></td>
<td><strong>Positive</strong></td>
</tr>
<tr>
<td><strong>Capillary voltage</strong></td>
<td>+ 4000 V</td>
</tr>
<tr>
<td><strong>Nebulizer pressure</strong></td>
<td>40 psi</td>
</tr>
<tr>
<td><strong>Drying gas flow</strong></td>
<td>13.0 L/min</td>
</tr>
<tr>
<td><strong>Drying gas temp.</strong></td>
<td>350 °C</td>
</tr>
<tr>
<td><strong>Mass range</strong></td>
<td>100-1600 (m/z)</td>
</tr>
<tr>
<td><strong>Fragmentor voltage</strong></td>
<td>120 V</td>
</tr>
</tbody>
</table>
LC/TOF-MS Chromatogram of a blood extract

Phenazepam
MW= 347.9665

Phenazepam
Rt= 19.399 min
LC/TOF-MS Spectrum of Phenazepam in blood extract

Phenazepam
Formula = C_{15}H_{10}BrClN_{2}O
Measured mass = 347.9671
Nominal mass = 347.9665
Mass Accuracy (ppm) = -0.26

Characteristic isotopic pattern for compounds with Cl and Br atoms
LC/TOF-MS Chromatogram of a urine extract

Methoxetamine
MW = 247.1572

Methoxetamine
Rt = 12.095 min
LC/TOF-MS Spectrum of Methoxetamine in a urine extract

Methoxetamine
Formula = C₁₅H₂₁NO₂
Measured mass = 247.1571
Nominal mass = 247.1572
Mass accuracy (ppm) = -0.42
# Summary of findings

## GC/MS:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Rt (min)</th>
<th>MW</th>
<th>Major ion Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenazepam</td>
<td>20.6</td>
<td>348</td>
<td>321, 350, 102, 75, 89, 177</td>
</tr>
<tr>
<td>Methoxetamine</td>
<td>13.3</td>
<td>247</td>
<td>190, 219, 134, 176</td>
</tr>
</tbody>
</table>

## LC/TOF-MS:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Rt (min)</th>
<th>MW</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenazepam</td>
<td>19.4</td>
<td>347.9665</td>
<td>$\text{C}<em>{15}\text{H}</em>{10}\text{BrClN}_{2}\text{O}$</td>
</tr>
<tr>
<td>Methoxetamine</td>
<td>11.8</td>
<td>247.1572</td>
<td>$\text{C}<em>{15}\text{H}</em>{21}\text{NO}_{2}$</td>
</tr>
</tbody>
</table>
Final Toxicology Results

1st Case:
- Blood
  - Lignocaine
  - Nevirapine
  - Phenazepam
- Urine
  - Lignocaine
  - Nevirapine
  - Phenazepam
  - Methoxetamine

2nd Case:
- Blood
  - Phenazepam
- Urine
  - Ephedrine
  - Phenazepam
  - Methoxetamine
Conclusion

• **Methoxetamine** is first time being reported in Singapore
• No literature being reported.
Acknowledgement

• Prof. Dr. Hans H. Maurer
  Department Head, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Germany

• Dr Yao Yi Ju
  Laboratory Director, Analytical Toxicology Laboratory, Clinical & Forensic Toxicology unit

• Alex Low Xuan Kai
  Scientist, Analytical Toxicology Laboratory, Clinical & Forensic Toxicology unit

• Tan Ying Ying
  Senior Scientist, Illicit Drug Laboratory

• All staff of the Analytical Toxicology Laboratory, Clinical & Forensic Toxicology unit
References


Thank You!  
Nadia_Wong@hsa.gov.sg