FACT SHEET

Methylnone

November 2013

For more information, please contact:
Dr. P. Blanckaert
Coordinator Belgian Early Warning System Drugs
Scientific Institute of Public Health
National Focal Point on Drugs
Jyllette Wytsmanstraat 14
B-1050 Brussels, Belgium
Tel : 02/642 5408
bewsd@wiv-isp.be
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A. General information

Recent collected sample in Belgium

Substance: methylone
Sample collected by Modus Vivendi, November 2013
Product type: Crystals
Color: white
Region: Brussels

Created
June 2005

Updated
October 2013

Type
Psychotropic substances

Group
Cathinones

Name
Methylone / bk-MDMA

Nature of substance
Methylone is the cathinone derivate of 3,4-methylenedioxymethamphetamine (MDMA). Methylone and related compounds can be described as ring-substituted cathinones, where cathinone, the parent compound and a scheduled drug in the 1971 UN Convention on Psychotropic Substances, is closely related to the phenethylamine family and an active constituent of khat. Methylone was reported by the Swedish and the Dutch NFPs in the beginning of 2005.

Systematic chemical name
3,4-methylenedioxymethcathinone

Other names
MDMCAT (preferred), MDMC
'Explosion', 'Room odorizer vanilla' (in the Netherlands). Methylone product sold under the name 'Inpact' may also be available in Japan.

B. Alerts

Alerts

Methylone related death, Hungary, January 2012
A fatal case related to the consumption of methylone, a relatively new cathinone type designer drug, was reported in Hungary. A 16-year-old boy suddenly lost his
consciousness in a party. Resuscitation had been continued for about 1.5 hours at the intensive care unit, but it was unsuccessful. His previous history included cardiac malformation detected at infancy and bronchial asthma had been diagnosed one year before his death. Signs of sudden cardiac death were observed during autopsy. Methylone intake was proved in blood and liver extract using gas chromatography/mass spectrometry; its concentration was 272 ng/ml in the blood, and 387 ng/g in the liver. Pathohistology revealed microvascular steatosis in the liver, which raised the possibility of chronic use of toxic substances. In addition, striated heart muscle damage was observed, which could be due to the use of an amphetamine-like substance. The authors presume that steatosis of the heart muscle, congenital heart disease and bronchial asthma could be predisposing factors for sudden cardiac death that occurred in the presence of relatively low levels of methylone. Access to various designer drugs is easy, fast and broad. Consequently, the potential abuse or overdose should be taken into consideration in the emergency practice. The use of "non-illicit" drugs does not require formal intervention by the authorities, but the medical service must alarm the stakeholders. Orv. Hetil., 2012, 153, 271-276. | doi:10.1556/OH.2012.29310"

Reports to EMCDDA
Numerous occasions of seizures, both large and small amounts, have been reported by nearly all member states in the European Union. Methylone was first identified by the Dutch national focal point in February 2005; its appearance has since spread throughout the European Union. The last seizure of methylone dates from August 2013 (Athens, Greece).

Seizures/collections in Belgium:
Belgium: On November 25th, the Belgian NFP reported the analysis of a collected sample (Modus Vivendi pill testing service) containing methylone.

Belgium: On 17 January 2013 the Belgian NFP reported several seizures of white powder (January 2012 (1kg), February (0.03 kg), June (1 kg), July (0.03 kg), August (1 kg), seized by customs at Brussels airport, sent from China.

Belgium: On 14 April 2010 the Belgian NFP informed that: 'a Belgian clinical laboratory reported the combined presence of mephedrone, butylone and methylone in a urine sample. The user declared to have bought it on the internet, as a plant food from the UK. The user said to have only used this one powder, so most likely, this powder contained all the three substances. More information about the effects could not be obtained, as the user left the hospital quite soon after the sample was taken.'
C. Pictures

Physical description
The substance has been encountered both, as liquid and tablet form: 5ml vials/tubes of 'room odorizer' in the Netherlands and white tablets in Sweden.

Powder found in an ‘amnesty bin’ in South Wales, UK in May 2008

D. Clinical information

Usage

Modes and scope of the established or expected use

Extent and frequency of use
There have been numerous seizures from European and neighbouring countries of methylone in powder, crystal, tablet, capsule and liquid form. There is no information on the purity of methylone in seizures or on adulterants/contaminants. There are currently no co-ordinated national or European population surveys on methylone use; however in the recent UK (2010) MixMag
survey of clubbers 10.8% of those surveyed had previously tried methylone and 7.5% of those surveyed had used methylone in the last month. Reports from the Swedish and the Dutch focal points mentioned that the drug was used as 'XTC'.

At the end of 2004, a product that contained methylone sold under the brand name 'Explosion' became available in some smart shops in the Netherlands. It was sold in tube-shaped bottles of liquid solution as a vanilla-scented "room odorizer". In early 2005, Explosion also became available for mailorder sale through Dutch smartshop online catalogs. The tubes costed between 10 and 15 Euro ($13 – $20) and did not present any information about the composition of Explosion; they contained only a label saying ‘Room odorizer Vanilla. Do not ingest’ and ‘Keep away from children. Never use more than one bottle’. In spite of this label, users mentioned that they ingested the liquid to reach the intended psychoactive effect. (The text was probably put onto the label to circumvent Dutch regulations for illicit drugs and psychoactive substances.)

**Routes of administration and dosage**
There are numerous user reports which detail routes of administration and dosage of methylone. The most common routes of administration described are oral ingestion and nasal insufflation, although there are occasional reports of rectal insertion and rarely of intravenous injection. Nasal irritation/pain may lead to some users changing from nasal insufflation to oral ingestion. The reported doses used varies widely, although most users report use of between 60-200mg of methylone.

**Onset of action and duration**
According to users on drug forums the peak duration of action for methylone is up to 3 hours with a longer elimination time.

**Subjective effects in man**
Described as “ecstasy for grownups” or “MDMA-lite” it offers elements of the ecstasy experience – physical and mental euphoria, increased energy levels, emotional insight and a lowering of social inhibitions – but at a lesser degree. Although user reports appear to suggest that methylone has similar properties to MDMA, they report that there are less stimulant effects seen with methylone in comparison. When methylone has been administered in vivo to rats trained to recognise and to distinguish the subjective effects of amphetamine, the animals weakly cross-generalised to methylone (as opposed to completely with methcathinone). However, methylone cross generalized completely to MDMA in rats trained to recognize this as the discriminative stimulus.

**Characteristics and behaviours of users / User groups and patterns**
A report [MacNamara et al., 2010] which examined a sample of methadone treated patients who attended a drug treatment centre in 2010 in Dublin found that those who were using “head shop” compounds like methylone were often abusing other substances also. Of the 29 out of 209 patients who tested positively for “head shop” compounds (mephedrone, methylone and BZP), 21 (41%) tested positive for Opiates, 21 (72.4%) tested positive for Benzodiazepines and 4 (13.8%) tested positive for Cocaine. Of the 29 positive for head shop compounds, 10 were tested for Amphetamines and 2 were positive. Methylone was detected in 3.3% of 209 urine
samples. This report also notes that methylone usage may have been underestimated
due to the fast metabolism of this substance.

Health risks
Commonly reported unwanted effects by users of methylone include difficulty
focusing, restlessness, change in perception of time, increase in body temperature,
increase in heart rate, muscle tension and aching, jaw tension, sweating, nausea and
vomiting, dizziness, confusion, paranoia. There are no reports on either user forums or
in the medical literature of chronic effects related to methylone use.
There are two published reports of presentations to healthcare services with acute
methylone health effects, only one of which has confirmation of methylone and its
major metabolites being present in urine. There have been a small number of calls to
poisons centres in the UK (n=7) and Sweden (n=4), but no clinical details are
available on these cases.

In January 2012, an alert was issued after the Hungarian national focal point reported
a death associated with the synthetic cathinone derivative methylone
(3,4-methylenedioxymethcathinone). The death involved a 16-year-old male.
Text below extracted from Bossong et al., 2005, see attached

Until now, no research has been conducted on the toxicity of methylone, so nothing is
known about the harmfulness of this new drug. Methylone resembles MDMA in its
behavioural profile, as methylone substitutes for MDMA in rats trained to
discriminate MDMA from saline. Methylone does not substitute for amphetamine or
for the hallucinogenic DOM in animals trained to discriminate between these drugs
and saline (Dal Cason et al., 1997). Further, also in common with MDMA, methylone
acts on monoaminergic systems. In vitro, methylone is threefold less potent than
MDMA at inhibiting platelet serotonin accumulation and as potent as MDMA in its
inhibiting effects on the dopamine and noradrenaline transporters (Cozzi et al.,
1999).

In spite of these behavioural and pharmacological similarities between methylone and
MDMA, the observed subjective effects of both drugs of abuse are not completely
identical (Erowid.org). Shulgin wrote about the effects of this drug: ‘methylone has
almost the same potency of MDMA, but it does not produce the same effects. It has an
almost antidepressant action, pleasant and positive, but not the unique magic of
MDMA’ (Cognitiveliberty.org).

Other uses
Unknown.

E. Legal status
Methylone is controlled throughout most of the European Union (controlled in Austra,
Bulgaria, Croatia, the Czech Republic, Denmark, Hungary, Ireland, Latvia, Poland,
Estonia, Finland, France, Germany, Lithuania, Solvakia, Romania, Slovenia, Sweden,
United Kingdom).

Not controlled in Belgium, Greece, or the Netherlands
F. Chemistry

Systematic chemical name
3,4-methylenedioxymethcathinone

Other chemical names and variants
2-methylamino-1-[3,4-methylenedioxyphenyl]propan-1-one (IUPAC)

Chemical Abstracts Service (CAS) registry number
Racemic Methylone: 186028-79-5; (S)-Methylone: 191916-41-3

Molecular information

Molecular structure

![Molecular structure of 3,4-methylenedioxymethcathinone](image)

Molecular formula: C11H13NO3

Molecular weight: 207.226

Synthesis, manufacture and precursors
No information.

Identification and analytical profile can be found at the end of this document and were kindly provided by the Irish Focal Point. This information is also available online.
G. References


Baumann M. H. et al., The Designer Methcathinone Analogs, Mephedrone and Methylone, are Substrates for Monoamine Transporters in Brain Tissue, Neuropsychopharmacology (2011), 1–12

ACMD report, Consideration of the cathinones, 31 March 2010

MacNamara S., Stokes S., Coleman N. (2010) Head shop compound abuse amongst attendees of The Drug Treatment Centre Board. Irish Medical Journal, 103 (5)


Simultaneous analysis of new designer drug, methylone, and its metabolites in urine by Gas Chromatography-Mass Spectrometry and liquid chromatography-electrospray ionization mass spectrometry

Inhibition of plasma membrane monoamine transporters by β-ketoamphetamines

Methylone and mCPP, two new drugs of abuse?
Bossong MG, Van Dijk JP, Niesink RJM, Addiction Biology 2005 December, 10, 321-323

Analytical profiles of the beta keto amphetamines (bkMDMA, bkMDEA, and bkMBDB)

The compounds

<table>
<thead>
<tr>
<th></th>
<th>Compound Description</th>
<th>Chemical Formula</th>
<th>Molecular Weight (fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one</td>
<td>C_{11}H_{13}NO_{3}</td>
<td>207.2</td>
</tr>
<tr>
<td></td>
<td>bkMDMA</td>
<td></td>
<td>‘Methyline’</td>
</tr>
<tr>
<td>B</td>
<td>2-ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one</td>
<td>C_{12}H_{15}NO_{3}</td>
<td>221.3</td>
</tr>
<tr>
<td></td>
<td>bkMDEA</td>
<td></td>
<td>‘Ethylone’</td>
</tr>
<tr>
<td>C</td>
<td>2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one</td>
<td>C_{12}H_{15}NO_{3}</td>
<td>221.3</td>
</tr>
<tr>
<td></td>
<td>bkMBDB</td>
<td></td>
<td>‘Butylone’</td>
</tr>
</tbody>
</table>

Controlled Class A

Not Controlled

Beta keto MDMA - Methylone

Beta keto MDEA - Ethylone

Beta keto MBDB – “Butylone”

*Butylone is also alleged to be trademarked name for pentobarbital*
**Methyelon**e is a beta-keto analogue of MDMA (Ecstasy). It is also known as bk-MDMA, M1, or MDMCat. Methyelon is more properly known as 3,4-methylenedioxymethcathinone and is related to methcathinone as MDMA is related to methamphetamine and MDA is to amphetamine.

In spite of some substantial pharmacokinetic differences (its dopaminergic activity is far more pronounced relative to its serotonergic activity), methyelon is an empathogen-like drug and a mild stimulant, producing effects similar to, yet less intense than MDMA.

Methyelon in powder formulation has recently (2008) been found in an amnesty bin from a UK dance venue.

**Ethylone** is the cathinone analogue of MDEA. It is reported to be less potent than methyelon and has only a short history of human use.

**bk-MBDB** is also known as "butylone": however Butylone is a trademarked name for pentobarbitone. Bk-MBDB shares the same relationship to MBDB as methyelon does to MDMA. The dosage range is not fully understood but seems to be lower than for MBDB. No formal research has been published on this substance, and nothing is known of its pharmacological profile or toxicology, although anecdotal reports indicate it is subjectively similar to but milder than methyelon.

Bk-MBDB has been found in tablets sold by UK based legal-high internet sites supplied by ‘London Underground’.

“Legal high” tablets such as those shown above are known to be inconsistent in their contents. It is possible that the same tablets bought at different times from the same site may differ in their contents.
GC/MS

Samples were analysed on a Shimadzu QP2010 gas chromatograph mass spectrometer with an HP5MS column (30m x 0.25mm, 0.50µm).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column oven temperature</td>
<td>80°C</td>
</tr>
<tr>
<td>Injection temperature</td>
<td>225°C</td>
</tr>
<tr>
<td>Injection mode</td>
<td>Splitless</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Helium</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 ml/min</td>
</tr>
<tr>
<td>Pressure</td>
<td>9.5 psi</td>
</tr>
<tr>
<td>Ion source temperature</td>
<td>200°C</td>
</tr>
<tr>
<td>Interface temperature</td>
<td>250°C</td>
</tr>
</tbody>
</table>

Column oven temperature programme:

<table>
<thead>
<tr>
<th>Rate</th>
<th>Final temperature</th>
<th>Hold time</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>80°C</td>
<td>4 minutes</td>
</tr>
<tr>
<td>40.00°C/min</td>
<td>280°C</td>
<td>8 minutes</td>
</tr>
<tr>
<td>40.00°C/min</td>
<td>290°C</td>
<td>11.5 minutes</td>
</tr>
</tbody>
</table>
### Chromatogram:

<table>
<thead>
<tr>
<th>ID</th>
<th>Compound Name</th>
<th>Abbreviations</th>
<th>Retention time (mins.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS-1</td>
<td>Quinoline</td>
<td>IS-1</td>
<td>8.819</td>
</tr>
<tr>
<td>A</td>
<td>2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one ‘Methylone’</td>
<td>bkMDMA</td>
<td>12.145</td>
</tr>
<tr>
<td>B</td>
<td>2-ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one ‘Ethylone’</td>
<td>bkMDEA</td>
<td>12.456</td>
</tr>
<tr>
<td>C</td>
<td>2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one ‘Butylone’</td>
<td>bkMBDB</td>
<td>12.546</td>
</tr>
<tr>
<td>IS-2</td>
<td>Pyribenzamine (tripelenamine)</td>
<td>IS-2</td>
<td>13.743</td>
</tr>
</tbody>
</table>
(A) 2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one
'Methyline' (bkMDMA) 12.145 mins

(B) 2-ethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one
'Ethylene' (bkMDEA) 12.456 mins

(C) 2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one
'Butylene' (bkMBDB) 12.546 mins

Susannah Davies
John Ramsey
7th August 2008
Analytical data

The product (1 mg) was extracted from basic (NH₃) aqueous solution/suspension (500 μl) into toluene (500 μl). The toluene extract and, in the case of dimethylamylamine which was derivatized by addition of an equal volume of pentafluoropropionic anhydride to the toluene extract and heating at 90°C for 20 minutes, evaporation and reconstitution in dichloromethane beforehand, was analysed by GCMS under the following conditions: Agilent 6890 gas chromatograph with split-splitless injection (2 μl injected) and a HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness). Helium (He) was used as the carrier gas at a flow rate of 1.0ml/minute. The GC was coupled to an Agilent 5973 MSD (EI, 70eV, TIC mode scanning m/z 40-800) and injector port was set at 300°C, the transfer line at 280°C, the ionization source at 220°C and the quadrupole at 150°C.

The following temperature program was used for mephedrone, methylene, flephedrone, ethcathinone, iso-ethcathinone, butylene, MDPV, naphyrone, dimethocaine, fluorotropococaine and 2-aminoindane: 90°C for 1 minute, 15°C/minute to 280°C, 280°C for 6.33 minutes, 10°C/minute to 300°C, 300°C for 13 minutes.

The following temperature program was used for dimethylamylamine (PFP derivative) and 2-phenylethylamine (PFP derivative): 50°C for 1 minute, 15°C/minute to 280°C, 280°C for 6.33 minutes, 10°C/minute to 300°C, 300°C for 10.34 minutes.

Samples were diluted appropriately if overloading was observed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>RT (min.)</th>
<th>RRT (relative to caffeine)</th>
<th>Ions (m/z) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mephedrone</td>
<td>177</td>
<td>7.35</td>
<td>0.70</td>
<td>58 (100.0), 91(13.7), 119(4.7), 162(0.4)</td>
</tr>
<tr>
<td>Methylene</td>
<td>207</td>
<td>9.42</td>
<td>0.90</td>
<td>58(100.0), 91(3.7), 121(5.9), 149(6.2)</td>
</tr>
<tr>
<td>Flephedrone</td>
<td>181</td>
<td>6.12</td>
<td>0.58</td>
<td>58(100.0), 75(17.1), 95(21.4), 123(5.3), 166(0.6)</td>
</tr>
<tr>
<td>Ethcathinone</td>
<td>177</td>
<td>6.83</td>
<td>0.65</td>
<td>72(100.0), 77(32.8), 105(9.7), 132(0.9)</td>
</tr>
<tr>
<td>‘iso-Ethcathinone’</td>
<td>177</td>
<td>6.42</td>
<td>0.61</td>
<td>79 (28.8), 104(9.5), 106 (9.4), 118(11.6), 134(100.0), 162(0.2), 176(0.2)</td>
</tr>
<tr>
<td>Butylene</td>
<td>221</td>
<td>9.94</td>
<td>0.95</td>
<td>72(100.0), 91(4.1), 121(10.5), 149(10.8)</td>
</tr>
<tr>
<td>MDPV</td>
<td>275</td>
<td>12.40</td>
<td>1.18</td>
<td>121 (9.7), 126(100.0), 149(10.9), 232(0.5)</td>
</tr>
<tr>
<td>Naphyrone</td>
<td>281</td>
<td>13.52</td>
<td>1.29</td>
<td>126(100.0), 155(4.6), 165(0.5), 238(0.5)</td>
</tr>
<tr>
<td>Dimethocaine</td>
<td>278</td>
<td>12.91</td>
<td>1.23</td>
<td>86(100.0), 120(18.9), 137(2.5), 278(0.7)</td>
</tr>
<tr>
<td>Fluorotropococaine</td>
<td>263</td>
<td>11.06</td>
<td>1.05</td>
<td>82(54.7), 95(54.1), 124(100.0), 140(6.0), 263(13.7)</td>
</tr>
<tr>
<td>2-Aminoindone</td>
<td>133</td>
<td>5.35</td>
<td>0.51</td>
<td>77(20.8), 91(57.8), 105(16.2), 116(57.2), 133(100.0)</td>
</tr>
<tr>
<td>Dimethylamylamine (PFP</td>
<td>115</td>
<td>5.35</td>
<td>0.45</td>
<td>119(22.5), 142(13.6), 164, (4.4), 190(100.0), 246(1.0)</td>
</tr>
<tr>
<td>derivative, diastereomers</td>
<td>and</td>
<td>5.97</td>
<td>0.46</td>
<td>and 119(21.3), 142(14.8), 164 (4.3), 190(100.0), 246(1.2)</td>
</tr>
<tr>
<td>observed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Phenylethylamine (PFP</td>
<td>121</td>
<td>8.64</td>
<td>0.66</td>
<td>91(67.0), 104(100.0), 119(20.7), 147(3.6), 176(10.4), 267(1.1)</td>
</tr>
<tr>
<td>derivative)</td>
<td>(PFP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>derivative)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A mass spectral library of the above compounds for Agilent Chemstation software is available on request (contact pierce.kavanagh@tcd.ie).

Standards of methylene, mephedrone, butylene, flephedrone, ethcathinone and MDPV were kindly provided by Ms. Sinéad McNamara (Drug treatment Centre Board, Dublin). Naphyrone was synthesized in our laboratory. 2-Aminoindane was purchased from Alfa Aesar (UK). Dimethylamylamine and 2-phenylethylamine were purchased from Sigma Aldrich (Ireland). Dimethocaine and fluorotropococaine were identified from their mass spectra as it is currently not possible to buy authentic standards from a reputable company. ‘iso-Ethcathinone’ was identified from mass spectral evidence as it is not commercially available. Our laboratory is currently in the process of synthesizing it.
Psychoactive “bath salts”: Not so soothing

Michael H. Baumann *, John S. Partilla, Kurt R. Lehner

Medicinal Chemistry Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 333 Cassell Drive, Suite 4500, Baltimore, MD 21224, USA

A R T I C L E   I N F O

Article history:
Received 21 August 2012
Received in revised form
4 November 2012
Accepted 14 November 2012
Available online 23 November 2012

Keywords:
Cathinone
Designer drug
Dopamine
Serotonin
Monoamine transporter

A B S T R A C T

Recently there has been a dramatic rise in the abuse of so-called “bath salts” products that are purchased as legal alternatives to illicit drugs like cocaine and 3,4-methylenedioxymethamphetamine (MDMA). Baths salts contain one or more synthetic derivatives of the naturally-occurring stimulant cathinone. Low doses of bath salts produce euphoria and increase alertness, but high doses or chronic use can cause serious adverse effects such as hallucinations, delirium, hyperthermia and tachycardia. Owing to the risks posed by bath salts, the governments of many countries have made certain cathinones illegal, namely: 4-methylmethcathinone (mephedrone), 3,4-methylenedioxymethcathinone (methylene) and 3,4-methylenedioxypprovalerone (MDPV). Similar to other psychomotor stimulants, synthetic cathinones target plasma membrane transporters for dopamine (i.e., DAT), norepinephrine (i.e., NET) and serotonin (i.e., SERT). Mephedrone and methylene act as non-selective transporter substrates, thereby stimulating non-exocytotic release of dopamine, norepinephrine and serotonin. By contrast, MDPV acts as a potent blocker at DAT and NET, with little effect at SERT. Administration of mephedrone or methylene to rats increases extracellular concentrations of dopamine and serotonin in the brain, analogous to the effects of MDMA. Not surprisingly, synthetic cathinones elicit locomotor activation in rodents. Stimulation of dopamine transmission by synthetic cathinones predicts a high potential for addiction and may underlie clinical adverse effects. As popular synthetic cathinones are rendered illegal, new replacement cathinones are appearing in the marketplace. More research on the pharmacology and toxicology of abused cathinones is needed to inform public health policy and develop strategies for treating medical consequence of bath salts abuse.

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1. “Bath salts” products contain synthetic cathinones

In the past few years, there has been an alarming increase in the abuse of so-called “bath salts” products sold on the internet and in retail shops. These products have no legitimate use as bath additives. Instead, they are purchased as “legal highs” that mimic the effects of illicit drugs like cocaine and 3,4-methylenedioxymethamphetamine (MDMA). Baths salts contain one or more synthetic derivatives of the naturally-occurring stimulant cathinone. Low doses of bath salts produce euphoria and increase alertness, but high doses or chronic use can cause serious adverse effects such as hallucinations, delirium, hyperthermia and tachycardia. Owing to the risks posed by bath salts, the governments of many countries have made certain cathinones illegal, namely: 4-methylmethcathinone (mephedrone), 3,4-methylenedioxymethcathinone (methylene) and 3,4-methylenedioxypprovalerone (MDPV). Similar to other psychomotor stimulants, synthetic cathinones target plasma membrane transporters for dopamine (i.e., DAT), norepinephrine (i.e., NET) and serotonin (i.e., SERT). Mephedrone and methylene act as non-selective transporter substrates, thereby stimulating non-exocytotic release of dopamine, norepinephrine and serotonin. By contrast, MDPV acts as a potent blocker at DAT and NET, with little effect at SERT. Administration of mephedrone or methylene to rats increases extracellular concentrations of dopamine and serotonin in the brain, analogous to the effects of MDMA. Not surprisingly, synthetic cathinones elicit locomotor activation in rodents. Stimulation of dopamine transmission by synthetic cathinones predicts a high potential for addiction and may underlie clinical adverse effects. As popular synthetic cathinones are rendered illegal, new replacement cathinones are appearing in the marketplace. More research on the pharmacology and toxicology of abused cathinones is needed to inform public health policy and develop strategies for treating medical consequence of bath salts abuse.

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ÒOwing to public health risk posed by bath salts, the governments of many countries have passed laws to render mephedrone, methylene, and MDPV illegal [Drug Enforcement Administration (DEA), 2011; Schifano et al., 2011]. Unfortunately, a new wave of cathinone derivatives has appeared in the marketplace to replace those drugs now subject to regulatory control (Brandt et al., 2010; Shanks et al., 2012), and the introduction of “replacement” cathinones is expected to continue.£

2. Bath salts cathinones target monoamine transporters

Despite the widespread use of bath salts, there is limited information about the mechanism of action underlying the physiological and behavioral effects produced by most synthetic cathinone derivatives. Emerging evidence indicates that bath salts cathinones interact with plasma membrane transporters for dopamine (i.e., DAT), norepinephrine (i.e., NET) and serotonin (i.e., SERT) (Baumann et al., in press; Sogawa et al., 2011). Data from our laboratory, summarized in Table 1, confirm that mephedrone and methylene block the uptake of $[^3H]dopamine$, $[^3H]norepinephrine$ and $[^3H]serotonin$ into rat brain synaptosomes (Baumann et al., in press). However, it must be clarified that traditional uptake-inhibition assays cannot discriminate between drugs acting as transporter substrates versus those acting as blockers, since both types of drugs prevent the accumulation of $[^3H]neurotransmitters$ into tissue. To address this problem, we and others have developed in vitro release assays in rat brain synaptosomes which can distinguish between these two types of drugs (Nagai et al., 2007; Rothman and Baumann, 2003; Rothman et al., 2001).

Results from release assays reveal that mephedrone and methylene function as substrates at monoamine transporters, thereby stimulating the release of $[^3H]$-methyl-4-phenylpyridinium ($[^3H]MPP^+$) via DAT and NET, and release of $[^3H]serotonin$ via SERT (Baumann et al., 2012; Nagai et al., 2007). The data in Table 1 show that mephedrone, methylene, and MDMA are non-selective transporter substrates (i.e., non-selective releasers), while amphetamine is a selective substrate at DAT and NET. Mephedrone displays similar releasing potency at all three transporters and is about twice as potent as methylene. Mephedrone, methylene, MDMA, and amphetamine are fully efficacious in the release assays (i.e., $E_{max}$ close to 100%), while MDPV and cocaine are inactive as releasers. The findings from assays using synaptosomes are consistent with the evidence demonstrating mephedrone and methylene function as transportable substrates in assays utilizing transfected cells expressing human DAT, NET and SERT (Eshleman et al., unpublished; Simmler et al., in press).

Recent data from our laboratory and others reveal that MDPV displays a novel pharmacological profile when compared to other bath salts cathinones (Baumann et al., in press; Simmler et al., in press). Specifically, MDPV is a potent uptake blocker at DAT and NET with no measurable substrate activity (see Table 1). The transporter blocking properties of MDPV are analogous to those of the structurally-related compound pyrovalerone (Heron et al., 1994; Meltzer et al., 2006). When compared to the prototypical transporter blocker cocaine: MDPV is 50-fold more potent at DAT, 10-fold more potent at NET, and 10-fold less potent at SERT. Taken together, the in vitro results indicate that mephedrone and methylene are non-selective transporter substrates, whereas MDPV is a pure catecholamine-selective transporter blocker.

### Table 1

<table>
<thead>
<tr>
<th>Transporter</th>
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<tr>
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<td>MDPV</td>
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<td>SERT</td>
<td>Mephedrone</td>
<td>MDPV</td>
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Several studies have reported that mephedrone and methylene inhibit the uptake of monoamine neurotransmitters in brain tissue and in cells, suggesting these two cathinones function as transporter blockers (Cozzi et al., 1999; Hadlock et al., 2011; Lopez-Arnau et al., 2012; Martinez-Clemente et al., 2012; Simmler et al., in press). Despite the widespread use of bath salts, there is limited information about the mechanism of action underlying the physiological and behavioral effects produced by most synthetic cathinone derivatives. Emerging evidence indicates that bath salts cathinones interact with plasma membrane transporters for dopamine (i.e., DAT), norepinephrine (i.e., NET) and serotonin (i.e., SERT) (Baumann et al., in press; Sogawa et al., 2011). Data from our laboratory, summarized in Table 1, confirm that mephedrone and methylene block the uptake of $[^3H]dopamine$, $[^3H]norepinephrine$ and $[^3H]serotonin$ into rat brain synaptosomes (Baumann et al., in press). However, it must be clarified that traditional uptake-inhibition assays cannot discriminate between drugs acting as transporter substrates versus those acting as blockers, since both types of drugs prevent the accumulation of $[^3H]neurotransmitters$ into tissue. To address this problem, we and others have developed in vitro release assays in rat brain synaptosomes which can distinguish between these two types of drugs (Nagai et al., 2007; Rothman and Baumann, 2003; Rothman et al., 2001).

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3. Synthetic cathinones produce stimulant effects in animals

A number of studies have examined the in vivo pharmacology of baths salts compounds in rodent models, though the majority of available data pertains to the effects of mephedrone (Angoa-Perez et al., 2012; Baumann et al., 2012; Hadlock et al., 2011; Huang et al., 2012; Kehr et al., 2011; Lisek et al., 2012; Lopez-Arnau et al., 2012; Marusich et al., 2012; Motbey et al., 2012). Because bath salts cathinones interact with monoamine transporters, they would be expected to increase extracellular concentrations of monoamine neurotransmitters in the brain. Consistent with this notion, in vivo microdialysis studies from our laboratory demonstrate that i.v. injection of mephedrone or methylone (0.3 or 1.0 mg/kg) increases extracellular levels of dopamine and serotonin in rat nucleus accumbens (Baumann et al., 2012). Kehr et al. (2011) and Wright et al. (2012) reported elevation of dialysate dopamine and serotonin in rat brain after subcutaneous (s.c.) mephedrone administration (3–10 mg/kg). Interestingly, the rise in extracellular serotonin is greater in magnitude than the rise in dopamine after mephedrone or methylone treatment, suggesting the neurochemical effects of both drugs are more akin to those produced by MDMA rather than methamphetamine (Baumann et al., 2012; Kehr et al., 2011; Wright et al., 2012). No microdialysis studies have examined the effects of cathinones on extracellular norepinephrine in the brain, and this issue warrants investigation. Mephedrone has a much faster rate of clearance when compared to MDMA, and this kinetic feature may increase the propensity for repeated binge use of mephedrone (Kehr et al., 2011).

Several investigations have reported that mephedrone produces locomotor stimulant effects in rats (Baumann et al., 2012; Kehr et al., 2011; Lisek et al., 2012; Motbey et al., 2012) and mice (Angoa-Perez et al., 2012; Lopez-Arnau et al., 2012; Marusich et al., 2012). Based on locomotor activity measures in rats undergoing microdialysis, mephedrone is similar in potency to MDMA but about three-fold less potent than amphetamine or methamphetamine (Baumann et al., 2012; Kehr et al., 2011). Intraperitoneal (i.p.) administration of mephedrone to rats (3, 5, 10 or 30 mg/kg) stimulates locomotor activity which is reversed by the dopamine-1 receptor antagonist SCH23390 (Lisek et al., 2012). Lopez-Arnau et al. (2012) reported that administration of mephedrone, methylone, or the related compound butylone (5, 10 or 25 mg/kg, i.p.), elicits dose-dependent hyperactivity in mice, and these effects are antagonized by pretreatment with the serotonin-2 receptor blocker ketanserin or the dopamine-2 receptor blocker haloperidol.

The fact that synthetic cathinones stimulate dopamine transmission predicts the drugs possess high abuse liability (Howell and Kimmel, 2008; Wise, 2008). Administration of mephedrone to rats (15 or 30 mg/kg, i.p.) produces a robust increase in the expression of Fos protein in reward-relevant brain regions such as the prefrontal cortex, ventral striatum, and ventral tegmental area (Motbey et al., 2012). In mice and rats, mephedrone (30 mg/kg, i.p.) engenders a positive place preference, which points to rewarding properties of the drug (Lisek et al., 2012). Robinson et al. (2012) showed that mephedrone administration (1, 3 or 10 mg/kg, i.p.) lowers brain stimulation reward thresholds in mice, and this effect is mimicked by identical doses of cocaine. Importantly, Hadlock et al. (2011) reported that i.v. mephedrone (0.24 mg/infusion) is self-administered by rats in a manner analogous to methamphetamine. Although less information is available about MDPV, one recent study demonstrated that i.v. MDPV (0.05, 0.1 or 0.2 mg/kg) is readily self-administered by rats, and when rats are allowed extended access to the drug, escalation of drug-taking behavior is observed (Watterson et al., in press). The collective findings provide compelling evidence that mephedrone and MDPV have a substantial propensity for addiction.

4. Toxicity and adverse effects

Serotonin transporter substrates like MDMA can produce sustained deficits in brain serotonin neurons (Baumann et al., 2007; Fleckenstein et al., 2007), so mephedrone and methylone might be predicted to have similar actions. Binge administration of either drug to single-housed rats (3 or 10 mg/kg, s.c., 3 doses) has no long-lasting effects on brain tissue monoamines (Baumann et al., 2012), while administration of higher doses of mephedrone to group-housed rats (10 or 25 mg/kg, s.c., 4 doses) produces persistent depletion of brain serotonin (Hadlock et al., 2011). The preclinical findings suggest that adverse effects of bath salts could be exacerbated in hot crowded conditions, such as those typical of rave dance parties where these drugs are sometimes ingested.

Patients coming to medical attention with bath salts intoxication can display agitation, combative behavior, psychosis, tachycardia, and hyperthermia (Borek and Holstege, 2012; Kyle et al.,...
et al., 2010. Health care workers should be cognizant that patients presenting with this constellation of symptoms may have taken bath salts. Because synthetic cathinones are not detected by routine toxicology screens, definitive proof of bath salts exposure is often difficult to confirm. Treatment is primarily supportive, with benzodiazepines such as lorazepam for agitation and excessive sympathetic stimulation, and aggressive cooling for severe hyperthermia (Ross et al., 2011; Spiller et al., 2011). In some instances, risperidone has been used effectively to manage psychotic behaviors, and in a single reported case, etomidate and succinyl choline were administered in addition to midazolam to sedate the patient (Kвисic et al., 2012; Antoniwiicz et al., 2011; Penders et al., 2012; Borek and Holstege, 2012).

MDPV and mephedrone have been directly implicated in a number of fatalities reported in the medical literature (Lusthof et al., 2011; Maskell et al., 2011; Murray et al., 2012). In one case involving MDPV (Murray et al., 2012), the cause of death was consistent with excited delirium syndrome, a condition that is associated with stimulant drug overdose and attributable to excessive dopaminergic transmission (Mash et al., 2009; Ruttenber et al., 1997). Symptoms of excited delirium include agitation, delirium, acidosis, sustained hyperthermia and autonomic dysfunction. Excited delirium has also been observed in bath salts overdose patients with analytical confirmation of mephedrone consumption (Kвисic et al., 2012; Lusthof et al., 2011). In a notable example, Kвисic et al. (2012) described a patient who had taken bath salts and was hallucinating, agitated, and hyperthermic; urinalysis revealed a presumptive positive for phencyclidine (PCP) as well as mephedrone (Kвисic et al., 2012). Interestingly, it was recently shown that MDPV cross-reacts with the PCP immunoassay used in hospitals (Macher and Penders, in press), suggesting the possibility that the patient described by Kвисic et al. ingested MDPV in combination with mephedrone. Penders et al. (2012) have concluded that MDPV is the most likely culprit responsible for causing excited delirium in patients who abuse bath salts products in the US. Because symptoms of excited delirium can be life-threatening, proper patient care is paramount. Physical restraints should be avoided if possible, and pharmacological treatment of agitation or cardiovascular symptoms should be administered prudently and monitored closely.

5. Summary

Psychoactive “bath salts” contain one or more synthetic cathinones which target plasma membrane monoamine transporters. In vitro data have identified a mechanistic dichotomy among common bath salts constituents: ring-substituted cathinones act as non-selective transporter substrates, whereas pyrrolidinophenones like MDPV act as potent catecholamine-selective transporter blockers (Baumann et al., in press; Nagai et al., 2007; Simmler et al., in press). Recent in vivo findings show that bath salts cathinones produce locomotor activation in rats and mice (Huang et al., 2012; Liske et al., 2012; Lopez-Arnau et al., 2012; Marusich et al., 2012), but few studies have examined pharmacokinetics and metabolism of synthetic cathinones (Kamata et al., 2006; Meyer et al., 2010), and the consequences of chronic drug dosing are unknown. The stimulation of dopamine transmission by bath salts cathinones is likely to mediate their abuse potential (Hadlock et al., 2011; Watterson et al., in press) and certain clinical side-effects (Murray et al., 2012; Penders et al., 2012). Given the emergence of new “replacement” cathinones with unknown pharmacology (Brandt et al., 2010; Shanks et al., 2012), it seems that emergency departments will continue to encounter patients suffering from the complications caused by synthetic stimulants. More research is urgently needed to characterize the pharmacology and toxicology of the growing list of synthetic cathinones. The data derived from these investigations will inform public health policy and improve strategies for treating the medical consequences of bath salts abuse.

Acknowledgments

The research described herein was generously supported by the Intramural Research Program at NIDA, NIH. The authors wish to thank Dr. Amy H. Newman for thoughtful comments on this manuscript.

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Case Report: Three Fatal Intoxications Due to Methylene

Julia M. Pearson1, *, Tiffany L. Hargraves2, Laura S. Hair1, Charles J. Massucci2, C. Clinton Frazee, III3, Uttam Garg4 and B. Robert Pietak5

1Hillsborough County Medical Examiner Department, Tampa, FL, 2University of Tampa Department of Chemistry and Physics, Tampa, FL, 3Tampa Police Department, Tampa, FL, 4Children’s Mercy Hospitals and Clinics, Kansas City, MO, and 5Jackson County Medical Examiner's Office, Kansas City, MO

*Author to whom correspondence should be addressed. Email: pearsonjm@hillsboroughcounty.org

We present three fatal intoxications of methylene, a cathinone derivative. Blood was analyzed with a routine alkaline liquid−liquid extraction and analyzed by gas chromatography coupled with a mass spectrometer (GC–MS). Methylene was identified by a full scan mass spectral comparison to an analytical standard of methylone. For a definitive and conclusive confirmation and quantitation, methylone was also derivatized with heptafluorobutyric anhydride and analyzed by GC–MS. In all three fatalities, the deceased exhibited seizure-like activity and elevated body temperatures (103.9, 105.9 and 107°F) before death. Two of the three cases also exhibited metabolic acidosis. One of the three cases had prolonged treatment and hospitalization before death with symptoms similar to sympathomimetic toxicity, including metabolic acidosis, rhabdomyolysis, acute renal failure and disseminated intravascular coagulation. The laboratory results for this patient over the 24 h period of hospitalization were significant for increased lactate, liver transaminases, creatinine, myoglobin, creatine kinase and clotting times, and decreased pH, glucose and calcium. Peripheral blood methylene concentrations in the three fatal cases were 0.84, 3.3 and 0.56 mg/L. In conclusion, peripheral blood methylene concentrations in excess of 0.5 mg/L may result in death due to its toxic properties, which can include elevated body temperature and other sympathomimetic-like symptoms.

Introduction

Cathinone, an alkaloid structurally similar to amphetamine, was originally extracted from the fresh leaves of the Catha edulis or khat plant, which is native to east Africa and the Arabian Peninsula (1). Synthetic structural modifications of cathinone have led to a number of so-called designer cathinone derivatives that are commonly sold as “bath salts” on the internet, in smoke shops or in specialty head shops. These cathinone derivatives, namely, mephedrone, methedrone, methylone and 3,4-methylenedioxypyrovalerone (MDPV), have become increasingly available and abused in the United States. The number of alarming case reports from emergency rooms, poison centers and medical examiners involving severe reactions, including death, has prompted several states and the federal government to ban or schedule the bath salts. In 2011, the American Association of Poison Control Centers (AAPCC) reported 6,072 bath salt–related calls, compared to only 303 in 2010 (2).

As with amphetamines, cathinones act as central nervous system stimulants by increasing monoamine neurotransmitter concentrations. This action is a result of at least two distinct mechanisms: one involving the inhibition of monoamine neurotransmitter reuptake and the other involving drug-evoked release of monoamine neurotransmitters from intracellular storage vesicles (3). Similar to the amphetamines, individual cathinones may vary in their potencies on each of the three monoamine neurotransmitter pathways. Methylene effects are similar to methamphetamine, with the greatest potency on the inhibition of noradrenergic reuptake, followed by inhibition of serotonergic and dopaminergic reuptake (3, 4). Similar to methamphetamine, methylene is also a potent releaser of intracellular dopamine, serotonin and norepinephrine (5).

Mild clinical effects of the cathinones include tachycardia, palpitations, agitation, lack of appetite, increased alertness, anxiety and mydriasis. Severe symptoms include seizures, hyperthermia, hallucinations, nausea and vomiting, muscle spasms, rhabdomyolysis, renal failure, arrhythmia and death (1, 6, 7, 8, 9). A handful of fatalities resulting from methedrone, mephedrone and MDPV toxicity have been reported; however, there is little information regarding methylene and its potential toxicity. In this case report, we present three fatalities involving methylene.

Case Histories

Case 1

At 5:30 p.m. on a Saturday evening, a 23-year-old male was walking in and out of traffic at a major intersection, banging on cars with fists, screaming profanities and acting erratically. He was yelling out rap song lyrics and speaking different languages. When approached by police, he refused to cooperate and became combative. He was taken down to the ground, subdued, handcuffed and transported to a local hospital emergency department. At the emergency room, he was combative, resisted removal from the police car and displayed unusual strength, requiring five individuals to help secure him to a hospital gurney.

Upon admission into the ER, he was restrained, combative and diaphoretic with a body temperature of 105.9°F. He was orally intubated for airway protection and sonorous respirations. Results from a computerized tomogram (CT) of the head and chest were unremarkable. He was bleeding from a tongue laceration (bite mark) that might have been caused from an acute seizure. He was placed under a cooling blanket and administered intravenous fluids containing antibiotics and pressors to treat possible septic shock and hypotension. Initial diagnoses were probable drug overdose, rhabdomyolysis, acute renal failure, acute respiratory failure, fever, confusional state and seizure. Approximately 3.5 h after admission, he went into cardiac arrest and, with cardiopulmonary resuscitation, was converted to sinus tachycardia. He went into cardiac arrest four more times over the next two hours. He developed disseminated intravascular coagulation (DIC), thrombocytopenia, anoxic encephalopathy and metabolic acidosis (without an
exhibited rigor. No admission blood or CT blood pressure of 63 responded and noted that the victim was seizing with an initial plastic food wrap and convulsing. 911 was called, paramedics located the victim. The victim was still bound to the chair with plastic food wrap. They wrapped the plastic food wrap around them and then placed the victim (in the chair) in the back of a van. 

Case 3
A 23-year-old male went out with friends to an after-hours club. A witness reported that the victim took LSD. The witness believed that the victim was having a "bad trip and freaking out." Another witness reported that the victim was acting "irrational and sweating." The victim also told a witness that he believed that the victim was having a "bad trip and freaking out." Another witness reported that the victim was acting "irrational and sweating." The victim was witnessed to take a pill known as "Molly." Shortly thereafter, she collapsed, and then got up and danced for several minutes. She then sat down, complaining of "not feeling right," and was witnessed to seize twice by an off-duty paramedic at the club. Each seizure was approximately 20 seconds long. Additional EMS personnel were called in, and the decedent was transported to a local hospital. She was in asystole upon arrival after having been given two units of epinephrine, two units of atropine and one dose of Narcan en route. Resuscitation efforts were continued for eight minutes after arrival. With no change in her condition she was pronounced dead. No signs of injury were noted and no froth cone was observed by hospital staff. While in the ER, her axillary temperature was 103.9°F and the code sheet indicated that she exhibited rigor. No admission blood or CT/X-ray scans were obtained. At autopsy, no extrinsic disease was noted.

Case 2
A 19-year-old female was reportedly attending a concert at a club in Kansas City, MO, with several friends. Around 1:00 a.m., she was witnessed to take a pill known as "Molly." Shortly thereafter, she collapsed, and then got up and danced for several minutes. She then sat down, complaining of "not feeling right," and was witnessed to seize twice by an off-duty paramedic at the club. Each seizure was approximately 20 seconds long. Additional EMS personnel were called in, and the decedent was transported to a local hospital. She was in asystole upon arrival after having been given two units of epinephrine, two units of atropine and one dose of Narcan en route. Resuscitation efforts were continued for eight minutes after arrival. With no change in her condition she was pronounced dead. No signs of injury were noted and no froth cone was observed by hospital staff. While in the ER, her axillary temperature was 103.9°F and the code sheet indicated that she exhibited rigor. No admission blood or CT/X-ray scans were obtained. At autopsy, no extrinsic disease was noted.

Experimental

Analysis
Methylenedioxyxymethylamphetamine (MDMA) was initially identified in all three cases while performing a routine alkaline drug screen by gas chromatography–mass spectrometry (GC–MS). An unidentified peak containing a prominent m/z 58 was identified between the retention times of cotinine and caffeine. This unidentified peak had a mass spectrum with a prominent base ion of 58 and low abundance ions of 91, 121 and 149, identical to the mass spectra for methylenedioxyxymethylamphetamine published in the March 2011 issue of ToxTalk (10). Subsequently, analytical standards for the bath salts, including methylenedioxyxymethylamphetamine, mephedrone, methedrone and MDPV, were purchased, spiked into blank blood, extracted and analyzed by GC–MS. The methylenedioxyxymethylamphetamine standard had the exact same retention time as the unidentified peak and an identical mass spectrum.

Due to the lack of any case reports or published methods in the scientific literature on methylenedioxyxymethylamphetamine and the nondescript mass spectrum of methylenedioxyxymethylamphetamine, a method was developed to confirm and quantitate methylenedioxyxymethylamphetamine using a heptfluorobutyryl (HFB) derivative to change the mass spectrum to a more unique spectrum and shift the retention time for a more definitive and conclusive identification of methylenedioxyxymethylamphetamine. In each case, methylenedioxyxymethylamphetamine was confirmed by full scan electron impact (EI) GC–MS mass spectral analysis of underivatized methylenedioxyxymethylamphetamine, full scan EI–GC–MS mass spectral analysis of the HFB derivative of methylenedioxyxymethylamphetamine and quantitated using the HFB derivative of methylenedioxyxymethylamphetamine. Figure 1 shows the EI mass spectrum for underivatized methylenedioxyxymethylamphetamine and Figure 2 shows the more unique mass spectrum of the HFB derivative of methylenedioxyxymethylamphetamine. Figure 3 shows the mass spectrum for the HFB derivative of the methylenedioxyxymethylamphetamine internal standard.

Reagents and materials
High-performance liquid chromatography (HPLC) grade toluene, hexane and ethyl acetate were supplied by Thermo Fisher Scientific. Heptfluorobutyryl anhydride (HFB) was supplied by Restek. Trisodium phosphate was supplied by Sigma-Aldrich Chemical Company. Analytical standards of methylenedioxyxymethylamphetamine and methylenedioxyxymethylamphetamine-d3 were supplied by Cerilliant. Enzyme-linked immunosorbent assay (ELISA) kits were supplied by Immunoassay.

Standards, calibrators and control preparation
An analytical standard solution (1 mg/mL) of methylenedioxyxymethylamphetamine was diluted to a working concentration (2 μg/mL) with methanol and stored at −10°C. Calibrators were prepared by spiking 1 mL blank blood to final concentrations of 0.05, 0.10, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/L methylenedioxyxymethylamphetamine. The internal standard, methylenedioxyxymethylamphetamine-d3, was spiked at a final concentration of 0.3 mg/L. Controls were prepared in 1 mL blank blood using a separate control working solution prepared from a separate batch of the same methylenedioxyxymethylamphetamine lot number at concentrations of 0.25, 0.75 and 1.5 mg/L.

Sample extraction and derivatization for confirmation and quantitation
The method developed is a slight modification of the standard amphetamine procedure at the Hillsborough County Medical
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</tr>
<tr>
<td>K (3.7–5.3) mEq/L</td>
<td>6.0</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
<td>7.2</td>
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<tr>
<td>Calcium (8.5–10.5) mg/dL</td>
<td>9.8</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
<td>8.1</td>
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<tr>
<td>Glucose (70–115) mg/dL</td>
<td>49</td>
<td>124</td>
<td>546</td>
<td>261</td>
<td>212</td>
<td>212</td>
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<tr>
<td>Magnesium (1.6–2.6) mg/dL</td>
<td>3.5</td>
<td>3.4</td>
<td>4.4</td>
<td>4.4</td>
<td>3.9</td>
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<tr>
<td>Phosphorus (2.7–4.5) mg/dL</td>
<td>9.1</td>
<td>16.0</td>
<td>20.5</td>
<td>20.5</td>
<td>20.5</td>
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<td>20.5</td>
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<tr>
<td>Lactic acid (0.5–2.2) mMol/L</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
<td>8.9</td>
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<td>8.9</td>
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<td>CO2 (21–31) mg/dL</td>
<td>19</td>
<td>18</td>
<td>20</td>
<td>20</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
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</tr>
<tr>
<td>BUN (6–20) mg/dL</td>
<td>18</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>17</td>
<td>17</td>
<td>17</td>
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<tr>
<td>Creatinine (0.5–1.2) mg/dL</td>
<td>2.7</td>
<td>4.2</td>
<td>4.0</td>
<td>4.0</td>
<td>5.0</td>
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<tr>
<td>BUN/creatinine ratio</td>
<td>6.7</td>
<td>6.2</td>
<td>6.0</td>
<td>6.0</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
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</tr>
<tr>
<td>Estimated GFR (mL/min)</td>
<td>29</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Osmolality (280–300) mOsm/kg</td>
<td>313</td>
<td>313</td>
<td>313</td>
<td>313</td>
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<td>313</td>
<td>313</td>
<td>313</td>
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<td>313</td>
</tr>
<tr>
<td>AST (5–40) IU/L</td>
<td>74</td>
<td>1,855</td>
<td>2,976</td>
<td>4,613</td>
<td>10,105</td>
<td>10,105</td>
<td>10,105</td>
<td>10,105</td>
<td>10,105</td>
<td>10,105</td>
</tr>
<tr>
<td>ALT (5–40) IU/L</td>
<td>40</td>
<td>1,515</td>
<td>3,406</td>
<td>6,196</td>
<td>5,132</td>
<td>5,132</td>
<td>5,132</td>
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<td>5,132</td>
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<tr>
<td>Total bilirubin (0.0–1.0) mg/dL</td>
<td>0.9</td>
<td>1.4</td>
<td>0.7</td>
<td>1.3</td>
<td>0.8</td>
<td>0.8</td>
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<tr>
<td>Alkaline phosphatase (39–117) IU/L</td>
<td>87</td>
<td>62</td>
<td>33</td>
<td>53</td>
<td>104</td>
<td>104</td>
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<tr>
<td>Total protein (6.1–7.9) g/dL</td>
<td>3.7</td>
<td>1.8</td>
<td>3.1</td>
<td>3.1</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Albumin (3.9–4.8) g/dL</td>
<td>4.7</td>
<td>1.7</td>
<td>&lt;1.0</td>
<td>1.5</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
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<tr>
<td>Anion gap</td>
<td>23</td>
<td>18</td>
<td>32</td>
<td>32</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
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<tr>
<td>Hemoglobin (14.1–18.1) gm/dL</td>
<td>9.0</td>
<td>11.7</td>
<td>9.4</td>
<td>8.2</td>
<td>5.4</td>
<td>5.4</td>
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<tr>
<td>White blood cell count (4.6–10.2) K/UL</td>
<td>5.17</td>
<td>1.80</td>
<td>1.96</td>
<td>2.61</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
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<td>1.90</td>
</tr>
<tr>
<td>Red blood cell count (4.6–8.13) M/UL</td>
<td>7.00</td>
<td>5.0</td>
<td>5.5</td>
<td>7.4</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Hematocrit (43.5–53.7) %</td>
<td>44.6</td>
<td>156</td>
<td>17.9</td>
<td>23.2</td>
<td>16.7</td>
<td>16.7</td>
<td>16.7</td>
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</tr>
<tr>
<td>Platelet count (142–424) K/UL</td>
<td>210</td>
<td>145</td>
<td>144</td>
<td>58</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Differential % granulocytes (30–77) %</td>
<td>70.2</td>
<td>68</td>
<td>59</td>
<td>75</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Differential % lymphocytes (15–47) %</td>
<td>22.9</td>
<td>20</td>
<td>23</td>
<td>17</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Differential % monocytes (3–13) %</td>
<td>5.7</td>
<td>10</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
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<tr>
<td>DMSB (&lt;5.9) ng/mL</td>
<td>17.3</td>
<td>17.3</td>
<td>17.3</td>
<td>17.3</td>
<td>17.3</td>
<td>17.3</td>
<td>17.3</td>
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<tr>
<td>Myoglobin (&lt;107) ng/mL</td>
<td>53,558</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
<td>&gt;500</td>
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<td>&gt;500</td>
</tr>
<tr>
<td>Troponin (0.4) ng/mL</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
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<td>0.18</td>
</tr>
<tr>
<td>CPK (&lt;171) IU/L</td>
<td>48,262</td>
<td>48,262</td>
<td>48,262</td>
<td>48,262</td>
<td>48,262</td>
<td>48,262</td>
<td>48,262</td>
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<tr>
<td>C-reactive protein (0.0–12.9) s</td>
<td>11.3</td>
<td>83.1</td>
<td>22.7</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
<td>&gt;88.8</td>
</tr>
<tr>
<td>APTT (210–340) s</td>
<td>21.5</td>
<td>&gt;170.0</td>
<td>1000</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
<td>&gt;130.0</td>
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</tbody>
</table>

*Normal ranges are expressed in parentheses.
Examiner Department. It was developed to identify and quantify methylone, mephedrone, methedrone and MDPV bath salts simultaneously; however, for the purposes of this publication, we present only the methylone results. One-milliliter volumes of specimens were extracted with 2 mL saturated trisodium phosphate buffer and 1 mL toluene. Samples were rotated slowly for 1 h, followed by centrifugation to achieve layer separation. The upper organic layer was transferred to conical bottom test tubes and evaporated under nitrogen at 30°C. Samples were reconstituted in 0.5 mL hexane and 50 μL HFBA was added to each sample. Samples were derivatized in a heat block at 70°C for 1 h. After derivatization, samples were removed from the heat block and evaporated under nitrogen at 30°C. Samples were reconstituted in 1.0 mL of saturated trisodium phosphate buffer and 200 μL of ethyl acetate was added to each sample. Samples were vortexed at high speed for 20 seconds, the layers were allowed to separate and a portion (approximately 100 μL) of the upper ethyl acetate layer was transferred to autosampler vials for analysis by GC–MS.

**Instrumentation and chromatographic conditions**

A Thermo Scientific ISQ GC–MS equipped with an AS300 autosampler was used for analysis. Chromatograph separation was performed on an RTx-5MS (Crossbond 5% diphenyl–95% dimethylpolysiloxane) 0.25 μm × 0.25 mm i.d. × 30 m column from Restek. The injection port was set at 260°C (injection volume 2 μL in splitless mode). The initial oven temperature was 100°C, with no hold time, ramped at 12°C/min to 200°C, then ramped at 30°C/min to reach a final temperature of 300°C, which was held for 4 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The transfer line temperature was 285°C.

EI was used as the ionization mode with an ion source temperature of 300°C and operated in selected ion monitoring (SIM) mode. Ions monitored for the HFBA derivatives were methylone-HFB (m/z 254, 110) and methylone-d3-HFB (m/z 257, 113). Ions used for quantification are underlined. The methylone-HFB and methylone-d3-HFB only have two unique ions each; therefore, all positive methylone cases were also confirmed in full scan (both underivatized and derivatized with HFBA) without the addition of internal standard for definitive identification and confirmation.

**Method validation studies**

The method was validated to assess calibration model, limit of detection, limit of quantitation, accuracy, precision, carryover and specificity. The calibration model was established by preparing a set of seven spiked calibrators (final concentrations of...
Figure 2. GC–MS full mass spectrum of HFB derivative of methylone with base ion of 149 and other minor ions of 91, 121, 210 and 254.

Figure 3. GC–MS full mass spectrum of HFB derivative of methylone-d3 with base ion of 149 and other minor ions of 91, 121, 213 and 257.
0.10, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 mg/L) plus a zero calibrator (blank) in five separate runs over five separate days. The calibration model was determined to be linear (unweighted with origin ignored) over the range of 0.1 to 2 mg/L with average $r^2 = 0.998$. The limits of detection and quantitation were estimated by the lowest acceptable non-zero calibrator within 20% of the target concentration. The limit of detection was 0.05 mg/L and the limit of quantitation was 0.10 mg/L. Accuracy and precision were assessed by preparing and analyzing the low, medium and high controls (spiked at final concentrations of 0.25, 0.75 and 1.5 mg/L) in triplicate over five different days. The overall bias did not exceed 5% and the within-run or between-run precision did not exceed 10%. Carryover was assessed by running a blank matrix sample immediately following the highest calibrator. There was no carryover at 2.0 mg/L.

Interference and specificity studies were conducted by analyzing 10 different sources of blank blood (including some bloods from blood bank, antemortem blood, postmortem peripheral blood, postmortem heart blood and postmortem cavity liquid) without the addition of internal standard. No endogenous blood components interfered with the analysis or contributed more than 10% of the ion signal of the lowest calibrators. Specificity studies were conducted by spiking blank blood with a number of other commonly detected drugs at a high concentration. Specificity studies indicated no interferences from 62 of the most frequently detected drugs in toxicology casework, including amphetamines, benzodiazepines, opiates, cocaine and metabolites, sedatives, hypnotics and antidepressants. When various tissues were analyzed, matrix matched blank tissues were spiked with controls to ensure that there were no obvious matrix effects on the quantitation of tissues using the calibration curve prepared in blood.

**Results**

In Case 1, screening was performed on the hospital admission samples. No volatiles were detected. Immunoassay was negative for 12 classes of drugs (acetaminophen, barbiturates, benzodiazepines, cannabinoids, carisoprodol/meprobamate, cocaine metabolite, fentanyl, methadone, methamphetamine/MDMA, opiates, oxycodone and salicylates). Analysis of an alkaline extract by GC–MS detected methylone and caffeine. No other drugs were detected. The femoral blood concentration of lamotrigine was 2.5 mg/L. Femoral blood was submitted to Hillsborough County Medical Examiner’s Office for the quantitation of methylone with a resulting femoral blood methylone concentration of 3.3 mg/L. The medical examiner concluded that the cause of death was acute methylone intoxication and the manner of death was accidental.

Case 2 originated in the Kansas City Medical Examiner’s Office. No volatiles were detected. Immunoassay results were negative for nine classes of drugs (cocaine metabolite, cannabinoids, opiates, benzodiazepines, phencyclidine, amphetamines, barbiturates, methadone and propoxyphene). Analysis of an alkaline extract by GC–MS detected the presence of methylone and lamotrigine. No other drugs were detected. The femoral blood concentration of lamotrigine was 2.5 mg/L. Femoral blood was submitted to Hillsborough County Medical Examiner’s Office for the quantitation of methylone with a resulting femoral blood methylone concentration of 3.3 mg/L. The medical examiner concluded that the cause of death was acute methylone intoxication and the manner of death was accidental.

In Case 3, the peripheral blood ethanol concentration was 0.03 g/dL and the vitreous humor ethanol concentration was 0.01 g/dL. No other volatiles were detected. Immunoassay was negative for nine classes of drugs (acetaminophen, barbiturates, carisoprodol/meprobamate, cocaine metabolite, methadone, methamphetamine/MDMA, opiates, oxycodone and salicylates). Analysis of an alkaline extract by GC–MS detected methylone and caffeine. The midazolam, lorazepam and fentanyl were all administered by the hospital with therapeutic blood concentrations (0.020, 0.029 and 0.0021 mg/L, respectively). Based on history, additional targeted analyses for LSD, GHB and cannabinoids were performed and all findings were negative.

Case 3 had the following methylone concentrations: 0.56 mg/L peripheral blood, 0.58 mg/L heart blood, 0.92 mg/L vitreous humor, 4.5 mg/L gastric contents, 0.88 mg/Kg liver and 230 mg/L urine. The medical examiner concluded that the cause of death was methylone intoxication and the manner of death was undetermined (due to the possible deleterious effects resulting from the restraint of the individual to a chair with plastic food wrap for several hours).

The results of quantitative analyses for methylone for the three cases are summarized along with reported body temperatures in Table II.

**Table II**

<table>
<thead>
<tr>
<th>Case</th>
<th>Body temperature (°F)</th>
<th>Methylone concentrations (mg/L or mg/Kg)</th>
<th>Antemortem blood</th>
<th>Peripheral blood</th>
<th>Heart blood</th>
<th>Liver</th>
<th>Vitreous humor</th>
<th>Urine</th>
<th>Gastric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105.9</td>
<td>0.70</td>
<td>1.0</td>
<td>1.4</td>
<td>0.55</td>
<td>12</td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
<td>103.9</td>
<td>0.84</td>
<td>3.3</td>
<td>0.92</td>
<td>230</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>107</td>
<td>0.56</td>
<td>0.58</td>
<td>0.88</td>
<td>4.5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only a portion of total gastric contents were submitted for analysis so the methylone concentration in gastric contents has limited interpretive value.*
Discussion

Methyline was readily detected in all three cases by a routine alkaline drug screen by GC–MS. However, because the drug is so new and little is known about it, a second definitive and conclusive confirmation of methyline was performed by derivatization with HFB to change the mass spectrum and shift the retention time. This resulted in two unique and distinct methods for the confirmation of methyline, a protocol that is recommended in forensic toxicology whenever possible or practical.

An attempt was made to set up the quantitation method that was similar to our amphetamine method with the HFB derivative. During the method development phase, methyline was added to the amphetamine calibrators in an attempt to simultaneously quantitate amphetamines and methyline using both methamphetamine-d5 and MDMA-d5 as internal standards. However, methyline did not exhibit acceptable linearity and had poor precision and accuracy. After some experimenting, it was discovered that using methylene-d3 internal standard greatly improved linearity, accuracy and precision. Despite increasing HFBA concentrations, it was also discovered that the methyline procedure worked best when analyzed separately from the amphetamines and derivatized 30 degrees higher than the amphetamines. It is possible that there was some type of competition for derivatization between methyline and amphetamines when analyzed simultaneously.

Case 1 provided an extremely rare complete clinical course of pathophysiological changes resulting from methyline toxicity, including elevated body temperature, acidosis and the sequelae of renal failure, liver failure and disseminated intravascular coagulation. Usually, this type of information is not available for postmortem cases. Case 1 also had the great potential to provide some insight regarding the half-life and elimination rate of methyline with the sequential hospital samples drawn over the entire 24 h time period. However, upon analysis of those samples, the blood concentration did not change significantly over time. This could either be due to a long half-life or, more probably, because the patient’s kidneys and liver were no longer functioning well at that time (as demonstrated by clinical values in Table 1). In addition, the patient was receiving various fluids, drugs and blood products via five intravenous and intra-arterial catheters in the intensive care unit, so some of the hospital blood samples may be subject to dilution with artificial fluids. Dilution might also explain why the postmortem heart and peripheral blood specimens are slightly higher than the antemortem specimens. It is also possible that higher postmortem methyline concentrations may be a result of postmortem redistribution. Additional cases and results will be needed to assess both the elimination rate of the drug and whether it is subject to postmortem redistribution.

Interestingly, all three of our cases had elevated body temperature. In review of the scientific literature reporting fatalities involving other bath salts, information of body temperature is not typically available because subjects were either found dead or hospital records were not available. However, one reported fatality involving methedrone had a reported body temperature of 107°F and similar clinical course of multiple organ failure over the 16 h period preceding death (11). In a recent study, repeated administrations of methyline and mephedrone produced a dose-dependent hyperthermia in rats (12). Mechanistically, it would be interesting to know whether all bath salts consistently produce high body temperatures, and whether this is an unusual finding, or perhaps a prognostic indicator of bath salt toxicity.

In Case 2, it appears that a relatively short period of time occurred from the time of alleged drug ingestion to the reported seizures and death. Lamotrigine, which was also identified in this case, has been shown to inhibit the reuptake of dopamine, norepinephrine and serotonin (13). This raises the possibility that an additive or synergistic effect between methyline and lamotrigine may have contributed to the rapid death in this case. Case 2 also had the highest methyline concentration and the lowest body temperature. In the other two cases with higher body temperatures and lower blood methyline concentrations, the individuals survived for several hours following ingestion of the drug. The differences in blood concentrations and clinical course might arise from different mechanisms of methyline toxicity, one in which high doses of methyline results in an acute rapid death and another in which lower doses may cause a more subacute toxic manifestation that includes extremely elevated body temperatures followed by multiple organ failure.

At the time the first case was analyzed, there were no reported cases of methyline intoxications in the published literature. Recently, there was a case reporting seizures and hypotension resulting from ethicathinone and methedrone poisoning (8). The victim developed rhabdomyolysis and DIC, but survived. No toxicological examinations were performed. More recently, a fatality resulting from methyline and butylone was reported (14). Similar to our cases, the victim was febrile, comatose, tachycardic and hypertensive upon arrival and despite maximal supportive care, developed multi-organ failure and expired. Toxicology examinations qualitatively identified methyline and butylone in urine and pill capsules.

Reports of fatalities involving other bath salts with qualitative toxicological examinations have been reported. Scotland reported four fatalities with mephedrone blood concentrations of 0.98, 2.24, 0.13 and 0.24 mg/L (15, 16). The Netherlands reported a fatal mephedrone intoxication with a blood concentration of 5.4 mg/L (17). Sweden reported two fatal mephedrone intoxications with blood concentrations of 15 and 9 mg/kg (11). Two abstracts presented at the 2011 Society of Forensic Toxicologists/The International Association of Forensic Toxicologist Joint Annual Meeting reported two fatal MDPV intoxications with blood concentrations of 0.44 and 1.0 mg/L, respectively (18, 19). This information on other bath salt cases was invaluable for the interpretation of the methyline concentrations in our cases.

As with all postmortem toxicology interpretations, the conclusions that these three cases resulted from methyline toxicity were based on the lack of any significant anatomical findings, individual case history and blood concentrations similar to other fatalities involving mephedrone, methedrone and MDPV. The methyline concentrations in these cases should assist in the interpretation of blood concentrations in other methyline-related cases.
References


Lethal Serotonin Syndrome After Methylone and Butylone Ingestion

Brandon J. Warrick · John Wilson · Matthew Hedge · Scott Freeman · Karen Leonard · Cynthia Aaron

Abstract

Introduction A new generation of designer phenethylamines have emerged and aggressively marketed as “legal highs.” The drugs are labeled “not for human consumption” to avoid widespread recognition and prosecution under the existing analog drug laws. The newest generation includes methylone and butylone. Methylone and butylone have minor structural changes and similar pharmacodynamics properties to scheduled drugs. Case Report We report a case of a healthy 24-year-old who ingested a capsule containing methylone and butylone sold as “Ecstasy” at a concert. The patient presented to the emergency department, comatose febrile, tachycardic, tachypnic, and hypertensive. On exam, she was diaphoretic, tremulous, hyperreflexic, and had sustained clonus. The patient was aggressively cooled, and despite maximal supportive care, the patient progressed to multi-system organ failure and ultimately expired. We obtained and analyzed both her urine and a capsule found on her person similar to the capsules ingested. In both samples, laboratory analysis identified only methylone and butylone.

Conclusion This is the first reported death for methylone or butylone and the first human or animal ingestion of butylone. Clinicians and public health officials should work together as new designer drugs emerge.

Keywords Methylone · Butylone · Bath salts · Synthetic phenethylamines · Intoxication · Death

Introduction

A new generation of designer phenethylamines has emerged and was being aggressively marked as “legal alternatives” to “Ecstasy” [methyleneoxymethamphetamine (MDMA)] or methamphetamine. A significant increase in synthetic phenethylamine intoxications have been reported since 2010 [1, 2]. Included in synthetic phenethylamines is methylone and butylone. These substances are distributed primarily over the internet but frequently encountered in convenience stores and “head shops.” They have escaped prosecution by law enforcement and the DEA by labeling products “not intended for human consumption” and marketed as bath salts or other non-ingestable products [2]. More recently, the DEA has temporarily scheduled methylone as a schedule I agent.

We present a 24-year-old female who ingested two capsules containing methylone and butylone, sold as “Ecstasy” resulting in multi-organ failure and mortality. The available literature on methylone and butylone is also reviewed.

Case Report

A healthy 24-year-old female with a history of psoriasis presented to the ED after ingesting two capsules of
“Ecstasy” at a concert (Fig. 1). Medics found her unconscious with a heart rate of 132 and a blood pressure 80/60 mmHg. EMS administered 10 mg of intravenous diazepam for apparent seizure activity. Although she experimented with marijuana and cocaine in the past, she was not a chronic abuser.

In the ED, the patient was nonverbal and withdrew to pain. Temperature was 41.8°C orally, pulse 158 bpm, respirations 34 bpm, blood pressure of 101/61 mmHg, and O₂ saturation of 100% on 100% O₂. Pupils were 6 mm and minimally reactive; mucus membranes were moist with a mild increase in secretions. Other than tachycardia and tachypnea, the heart, lungs, and abdomen were unremarkable. Skin was hot and diaphoretic. The patient was comatose, withdrawing symmetrically to pain, did not open her eyes, but did make incomprehensible sounds. Tremors were noted in the upper extremities with increased tone and bilateral ankle clonus.

She was fluid resuscitated, mechanically ventilated, and externally cooled with ice packs. Myoclonus was initially controlled by 2 mg of intravenous lorazepam followed by 32 mg of midazolam administered between 15 and 45 min post presentation. Although benzodiazepines decreased myoclonus, they did not fully extinguish it. Non-depolarizing neuromuscular blockers were used to stop all muscle activity to control the hyperthermia. Three hours after neuromuscular blockade, core temperature decreased to 37.7°C. Four hours after arrival, vital signs were BP 147/83 mmHg, pulse of 130, and temperature of 37.1°C. No additional paralytics were given.

Initial ECG showed sinus tachycardia with a rate of 159 bpm, QRS 108 ms, QTc 488 ms, without ischemic changes. Initial laboratory results are detailed in Table 1. Urine for GC–MS was sent but results were unavailable for 24 h.

Shortly after hyperthermia was controlled, she developed posterior epistaxis and oozing from IV sites. Repeat coagulation tests were consistent with DIC. A posterior nasal pack was placed and she was transfused with fresh frozen plasma, packed red blood cells, and platelets to obtain hemostasis.

Ten hours post-admission, the patient developed pulseless electrical activity arrest from an unclear etiology. Spontaneous circulation returned within 90 s of CPR and 1 mg of epinephrine, but she required multiple pressors (norepinephrine, vasopressin, and dopamine). On repeat examination, clonus, hyperreflexia, and tremor had resolved. Her coagulopathy did not respond to treatment. Repeat lab values showed multisystem organ failure (Table 1). Given the poor prognosis, the family and care team agreed to withhold CPR if the patient’s condition deteriorated.

Over the next 24 h, her hemodynamics and coagulopathy improved. However, she developed ARDS and renal failure. Despite optimal ventilation, hypoxemia, and lactic acidosis worsened and she expired 48 h after presentation.

### Drug Analysis

The patient purchased multiple capsules and ingested two capsules according to her friends. A capsule found in the patient’s possession and her urine were analyzed and compared to pure analytical reference standards obtained from Cerilliant Corporation (Catalog #M-140, B-045 Round

<table>
<thead>
<tr>
<th>Table 1 Laboratory results</th>
<th>Initial</th>
<th>12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.31</td>
<td>7.49</td>
</tr>
<tr>
<td>pCO₂</td>
<td>35 mmHg</td>
<td>30 mmHg</td>
</tr>
<tr>
<td>pO₂</td>
<td>230 mmHg</td>
<td>67.6 mmHg</td>
</tr>
<tr>
<td>Na</td>
<td>140 mEq/L</td>
<td>148 mEq/L</td>
</tr>
<tr>
<td>K</td>
<td>5.0 mEq/L</td>
<td>2.4 mEq/L</td>
</tr>
<tr>
<td>Cl</td>
<td>105 mEq/L</td>
<td>116 mEq/L</td>
</tr>
<tr>
<td>Bicarb</td>
<td>23 mEq/L</td>
<td>15 mEq/L</td>
</tr>
<tr>
<td>BUN</td>
<td>11 mg/dL</td>
<td>22 mg/dL</td>
</tr>
<tr>
<td>Cr</td>
<td>1.9 mg/dL</td>
<td>3.0 mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>198 mg/dL</td>
<td>126 mg/dL</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>&lt;10 mcg/ml</td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>&lt;5 mg/dL</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>26 Units/L</td>
<td>366 Units/L</td>
</tr>
<tr>
<td>ALT</td>
<td>19 Units/L</td>
<td>138 Units/L</td>
</tr>
<tr>
<td>CPK</td>
<td>152 Units/L</td>
<td>926 Units/L</td>
</tr>
<tr>
<td>Troponin I</td>
<td>0.875 ng/mL</td>
<td>20.3 ng/mL</td>
</tr>
<tr>
<td>INR</td>
<td>1.04</td>
<td>&gt;11.49</td>
</tr>
<tr>
<td>APTT</td>
<td>23.6 s</td>
<td>&gt;200</td>
</tr>
<tr>
<td>WBC</td>
<td>9,800/mm³</td>
<td>5,500/mm³</td>
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<tr>
<td>Hemoglobin</td>
<td>14.5 g/dL</td>
<td>10.9 g/dL</td>
</tr>
<tr>
<td>Platelets</td>
<td>200,000/mm³</td>
<td>84,000/mm³</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Undetectable</td>
<td></td>
</tr>
</tbody>
</table>
Rock, TX, USA) at concentrations of 1 mg/mL in methanol. One-microliter sample injection provided the reference response. Two milliliters of the patient’s urine was extracted into dichloromethane at a basic pH, the organic fraction was removed and evaporated to dryness under an airstream at 40°C, and reconstituted with 25 μL of ethyl acetate. One microliter was injected into an Agilent 5972A MSD (Agilent, Andover PA, USA) GC/MS. A small portion of the submitted capsule was weighed and dissolved in 10 mL of methanol. One microliter was injected consistent with reference standards and patient samples and the instrument TIC response used to calculate the capsule dose by relative peak area.

The powder mass was 619 mg. Analysis revealed the 422 mg of methylone and 53 mg of butylone. GC/MS of the urine was positive for methylone and butylone and negative for other drugs of abuse including THC and cocaine. Autopsy revealed evidence of a generalized coagulopathy, fatty liver, and anoxic encephalopathy. According the medical examiner, the cause of death was serotonin syndrome secondary to methylone and butylone.

Discussion

This patient presented to the Emergency Department hyperadrenergic with serotonin excess. She was hyperthermic with uncontrolled muscle activity and possible seizure after ingesting two capsules sold as “Ecstasy.” Methylone and butylone were confirmed by laboratory analysis in the capsule and patient’s urine.

We reviewed the literature on methylone and butylone through PubMed, Google Scholar, and unorthodox websites, using the search terms methylone or butylone. Pharmacokinetics and pharmacodynamic information is minimal for methylone and we were unable to find any data on butylone. Methylone is the beta-keto analogue of methylenedioxymethamphetamine (MDMA) and referred as bk-MDMA or M1. Butylone is the beta-keto analogue of methylbenzodioxylbutanamine (MBDB) referred as bk-MBDB or B1 (Fig. 2). The only published dose of methylone was 120 mg mixed with 76 mg 5-MeO-MIPT (N-isopropyl-5-methoxy-N-methyl-tryptamine) [3]. On-line first person accounts indicate 100–250 mg is a common dose of methylone. A “heavy” dose is greater than 250 mg. Writers describe drug effect onset at 15–30 min with duration of 2–3.5 h but 6–24 h to return to “normal,” oral doses are slightly less effective than equivalent IV doses [4]. There are no published human ingestions of butylone in the medical literature. On-line bloggers report doses of 150–250 mg [4].

The pharmacodynamic literature is limited to one in vivo report, two case reports, and numerous “trip reports” for methylone [3, 5–7]. Similar to MDMA, MBDB, and other phenethylamines, methylone, and butylone are expected to increase levels of serotonin, dopamine, and norepinephrine [7]. In vitro, methylone is threefold less potent than MDMA at inhibiting platelet serotonin accumulation. Methylone is equipotent to MDMA for inhibition of dopamine and norepinephrine transporters [6]. Reports from on-line blogs and forums suggest the psychotropic effects of butylone and methylone are similar although users report the ability to differentiate butylone from methylone, MDMA and MBDB [4].

In both case reports involving methylone, the patients had euphoria, dilated pupils, and diaphoresis [3, 5]. Only one report provides a detailed physical examination consistent with serotonin excess complicated by a seizure [3]. The authors hypothesized the seizure resulted from hyponatremia secondary to water intoxication. Our case met the Hunter criteria for serotonin syndrome with hyperreflexia, tremor, muscle rigidity, spontaneous clonus, and temperature >38°C [8, 9]. Further supporting the diagnosis of serotonin syndrome is resolution of hyperreflexia and myoclonus within 24 h. Since it is not possible to differentiate the presence of excess dopamine or norepinephrine by

![Fig. 2 Beta-keto analogue of methylenedioxymethamphetamine (MDMA) and methylbenzodioxylbutanamine](image)
physical exam, we assume both cases had an excess of dopamine and norepinephrine based on consistency with in vivo data.

Our patient died after ingesting two capsules despite expeditious treatment. Based on analytic evidence from a single capsule, we suspect she ingested 844 mg of methylone plus 106 mg of butylone. The dose the patient ingested was substantially over what has been reported as a “normal” dose. However, we cannot confirm this dose since any illicitly marketed drug has substantial variability in purity and concentration. Another factor that may have contributed to her poor outcome was the unknown duration of symptoms prior to presentation to the hospital. Although cooling measures were immediately begun in the ED, the patient developed DIC and had a brief pulseless electrical activity arrest. While she was successfully resuscitated from the arrest, multi-organ system failure progressed despite aggressive supportive care.

Conclusion

Methylone and butylone are emerging drugs of abuse and this is the first report to highlight a death from either substance. While this case is fairly typical for severe serotonin syndrome and/or sympathetic intoxication, it highlights the potential lethality of these drugs. The case underlines our need to better understand the pharmacology mechanism of action, and optimal treatment of synthetic phenethylamine intoxication. While serving as surveillance for emerging drugs of abuse, poison centers need to proactively educate healthcare providers, public health officials, and the community regarding the dangers of these readily available designer phenethylamines.

References