Journal of Analytical Toxicology 2015;39:567–571
doi:10.1093/jat/bkv053 Advance Access publication May 29, 2015

Technical Note

Ethylone-Related Deaths: Toxicological Findings

Dayong Lee, Chris W. Chronister, Jennifer Hoyer and Bruce A. Goldberger*

Forensic Toxicology Laboratory, Division of Forensic Medicine, Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, 4800 SW 35th Drive, Gainesville, FL 32608, USA

*Author to whom correspondence should be addressed. Email: bruce-goldberger@ufl.edu

Synthetic cathinones are an emerging class of designer drugs, frequently with deceptive labels and a multitude of analogs to circumvent drug control regulations. Research regarding the pharmacological effects and toxicity of these amphetamine derivatives is scarce, heightening the risk to the public health and safety. The composition of synthetic cathinone products continually changes and laboratories began to notice ethylone-positive products in late 2011. This report presents nine postmortem cases in whom ethylone was identified. Ethylone was isolated using solid-phase extraction and detected by gas chromatography–mass spectrometry. Seven of the cases had measurable concentrations of ethylone in blood, ranging from 38 to 2,572 ng/mL; ethylone was detected in the blood sample of one case with a concentration below the assay limit of quantification (25 ng/mL), and one case did not have detectable ethylone in blood. Besides ethylone, all but one case were also positive for 11-nor-9-carboxy-Δ9-tetrahydrocannabinol; seven cases had other drugs quantified in blood, including ethanol, alprazolam, benzoylecgonine, diphenhydramine, morphine and tramadol. In five cases where ethylone was present at blood concentrations >400 ng/mL, no other drugs excluding ethanol, cannabis metabolite and doxylamine (one case) were found. The assay also tested for mephedrone, methylone and three dimethoxyamphetamine analogs; no case was positive for these analytes. The present report documents postmortem blood concentrations of ethylone, a novel synthetic cathinone, along with other concurrently identified substances. The findings provide valuable information for developing analytical assays and evaluating a toxic concentration range of ethylone.

Introduction

Synthetic cathinones are emerging drugs of abuse with central nervous system-stimulant properties similar to cocaine, 3,4-methylenedioxymethamphetamine (MDMA) and other conventional amphetamines (1, 2). These are β-keto amphetamine derivatives of cathinone, the principal psychoactive constituent in the plant *Catha edulis* (Khat) (3). Social media began alluding to synthetic cathinones in 2007 (1). In 2010, the American Association of Poison Control Centers (AAPCC) reported 303 calls related to synthetic cathinones; the number sharply increased to 6,137 calls in 2011 and declined to 996 in 2013 (4). Similarly, the National Forensic Laboratory Information System (NFLIS) initially reported 602 synthetic cathinone-positive cases in 2010, which increased to 6,542 in 2011; 7,997 cases had been recorded as of June 2013 (5).

The products are surreptitiously sold as 'bath salts', 'plant food' or 'research chemicals' and labeled 'not for human consumption' to evade drug control legislation (6). They can be obtained on the internet, in 'head shops' or 'smart shops', and also from local drug suppliers. The most prevalent drugs found in these synthetic cathinone products have been 3,4-methylenedioxy-*N*-methylcathinone (methylone), 3,4-methylenedioxypyrovalerone (MDPV), 4-methyl-*N*-methylcathinone (mephedrone) and more recently, α-pyrrolidinopentiophenone (α-PVP), 4-methylethcathinone (4-MEC), 2-(methylamino)-1-phenylpentan-1-one (pentedrone), and others have additionally been identified (5). However, the composition of synthetic cathinones on the clandestine drug market is continually transforming and new compounds have been manufactured via slight alterations in chemical structure to subvert existing regulations. In 2011, the US Drug Enforcement Administration categorized mephedrone, methylone and MDPV as Schedule I drugs (7). In 2014, 10 other synthetic cathinones were placed into Schedule I (8): 4-MEC, 4-methyl-α-pyrrolidinopropiophenone (4-MePPP), α-PVP, 1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one (butylone), pentedrone, 1-(1,3-benzodioxol-5-yl)-2-(methylamino)pentan-1-one (pentylone), 4-fluoro-*N*-methylcathinone (4-FMC), 3-fluoro-*N*-methylcathinone (3-FMC), 1-(naphthalen-2-yl)-2-(pyrrolidin-1-yl)pentan-1-one (naphyrone) and α-pyrrolidinobutiophenone (α-PBP).

Ethylone [1-(1,3-benzodioxol-5-yl)-2-(ethylamino)propan-1-one; 3,4-methylenedioxy-*N*-ethylcathinone, MDEC; β-keto-methylenedioxyethylamphetamine, bk-MDEA] is a newer, N-ethyl form of methylone (Figure 1). The NFLIS had not received ethylone-positive cases until the second half of 2011. From then to the first half of 2013, 105 ethylone-positive reports were submitted from Federal, state and local laboratories throughout the USA (5). Four of the 35 'bath salt' products purchased from California retail stores and the Internet in August–December 2011 contained 0.8–155 mg of ethylone (9).

An *in vitro* radioligand binding assay showed that ethylone nonselectively inhibits the monoamine transporters with potency comparable to or lower than that of cocaine (mean IC_{50}, 2.5–5.7); the drug also releases serotonin (mean EC_{50}, 9.9 μM) similar to MDMA and other entactogens (10). Ethylone is mainly metabolized via demethylenation of the methylenedioxy ring, followed by O-methylation and subsequent conjugation with glucuronic acids and/or sulfates; the minor pathways include N-deethylation and β-ketone reduction (11, 12). While acidic hydrolysis increased the concentrations of the phenolic metabolites (4-hydroxy-3-methoxy- and 3-hydroxy-4-methoxy-*N*-ethylcathinones), the parent compound seems to be the primary analyte in human urine (11, 13, 14). In another study, 34,561 random urine samples collected in 2011–2013 were analyzed for 16 synthetic cathinones, and 16 (0.05%) were positive for ethylone; α-PVP was the most prevalent (2.5%), followed by MDPV (1.7%) and pentedrone (1.2%) (14). When 325 hair samples from 2009 to 2010 initially positive for amphetamines and/or MDMA were reanalyzed for seven synthetic cathinones, ethylone was not identified at the limit of detection of 10 pg/mg; mephedrone was found

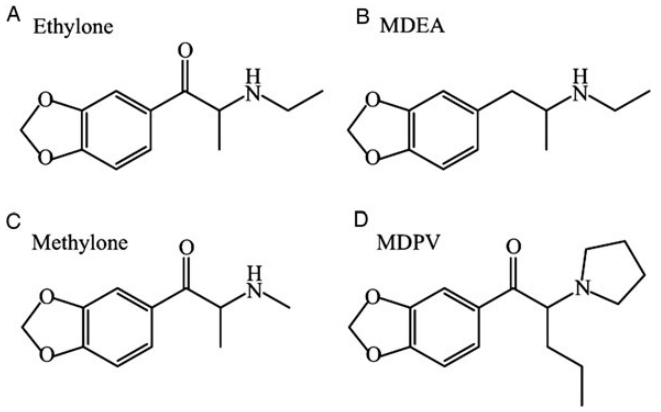

Figure 1. Chemical structure of ethylone (A), methylenedioxyethylamphetamine (MDEA; B), methylone (C) and methylenedioxypyrovalerone (MDPV; D).

in 11 samples and methylone in 1 sample (15). Recently, a suspected impaired driving case positive for ethylone, α-PVP and methylone was reported (16).

Research on synthetic cathinones in biological matrices is limited, especially in postmortem cases. Evaluation of ethylone disposition is even scarcer owing to its more recent appearance on the clandestine drug market. This study reports postmortem cases in 2014 that screened positive for ethylone in urine or bile samples; in eight of the cases, ethylone was detected in blood. This study provides valuable information pertaining to ethylone concentrations that could be potentially encountered during postmortem investigations. Furthermore, the composition of other drugs concurrently detected with ethylone is presented, capturing a glimpse of drug intake patterns in ethylone users.

Case description

The nine postmortem cases included in this report were submitted to the Forensic Toxicology Laboratory at the University of Florida from April to November 2014 for toxicological testing. The postmortem specimens were collected by the state of Florida district medical examiners. The decedents were all young males (18–32 years old). The decedents' demographic information and cause of death are indicated in Table I.

Table I
Summary of Demographics and Decedent Cause of Death

Case	Age	Sex	Race	Decedent cause of death
1	23	M	Black	Gunshot wound of chest
2	29	M	Black	Multiple gunshot wounds
3	25	M	White	Intoxication with alprazolam, cocaine and heroin
4	18	M	Black	Gunshot wound of chest
5	27	M	White	Undetermined
6	24	M	Black	Blunt impact to head and torso; contributing ethylone and ethanol intoxication
7	24	M	Black	Hanging
8	32	M	Black	Blunt force head trauma; contributing ethylone and ethanol intoxication
9	23	M	Black	Perforation of the left subclavian artery and vein and left common carotid due to gunshot wound of torso and neck

Methods

Reagents and materials

Ethylone, mephedrone, methylone, 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-chloro-2,5-dimethoxyamphetamine (DOC) and 4-iodo-2,5-dimethoxyamphetamine (DOI) were purchased from Cerilliant Corporation (Round Rock, TX, USA) along with the deuterated internal standards, ethylone-d_5, mephedrone-d_3, methylone-d_3 and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB)-d_5. Ethylone utilized in the preparation of positive control samples was purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

Ethyl acetate, isopropanol, methanol, ammonium hydroxide, glacial acetic acid, hydrochloric acid, methylene chloride, potassium hydroxide and potassium phosphate monobasic were purchased from Fisher Scientific (Pittsburgh, PA, USA); all solvents and reagents were ACS grade. Deionized water was obtained via the Millipore water purification system (Billerica, MA, USA). Pentafluoropropionic acid anhydride (PFPA) and trimethylanilinium hydroxide (TMAH) as MethElute Reagent were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Clean Screen® mixed-mode solid-phase extraction (SPE) columns (ZSDAU020) were acquired from United Chemical Technologies (Bristol, PA, USA).

Comprehensive drug screen

Testing for volatile compounds was performed utilizing automated headspace gas chromatography (GC) with flame ionization detection. An immunoassay screen of urine for amphetamines, benzodiazepines, cannabinoids, cocaine metabolite and opioids was also conducted utilizing CEDIA™ (Thermo Fisher, Freemont, CA, USA). An acid/base/neutral qualitative drug screen was performed using mixed-mode SPE. The acid extracts were derivatized with TMAH. All extracts were analyzed by GC-nitrogen phosphorus detection (NPD) and confirmed by GC–full scan mass spectrometry (MS).

Synthetic amphetamine confirmatory analysis

Working standard solutions of 1 and 10 μg/mL of ethylone, mephedrone, methylone, DOB, DOC and DOI as well as 10 μg/mL of ethylone-d_5, mephedrone-d_3, methylone-d_3 and MBDB-d_5 (as the internal standard for DOB, DOC and DOI) were prepared in methanol and stored at $\leq -10^{\circ}$C. Calibrators were prepared in 1.0 mL of drug-free human whole blood by fortifying the analytes at final concentrations of 25, 50, 100, 250 and 500 ng/mL. Negative control and blank samples were 1.0 mL of drug-free whole blood with and without 10 μL of 10 μg/mL the internal standard, respectively. Positive control samples were 1.0 mL of drug-free whole blood fortified with analytes at 100 ng/mL; separate sources of the analytes were used when preparing calibrator and control standard solutions. Blank, negative and positive controls as well as an unextracted standard (10 μL of 10 μg/mL standard solution and internal standard) were included in each batch.

Into 13 × 100 mm test tubes, 1.0 mL of case samples, calibrators and controls were pipetted and fortified with the internal standard to final ethylone-d_5, mephedrone-d_3, methylone-d_3 and MBDB-d_5 concentrations of 100 ng/mL. Two milliliters of 0.1 M phosphate buffer (pH 6) were added to all tubes, which were then vortexed and centrifuged at 1,500 × g for 5 min. Prior to loading the samples onto the Clean Screen® SPE columns placed in the positive pressure manifold, each column was rinsed

with 3 mL of methylene chloride : isopropanol : ammonium hydroxide (78 : 20 : 2, v/v/v) and conditioned sequentially with 3 mL of methanol and 2 mL of 0.1 M phosphate buffer (pH 6) at a flow rate of ~2 mL/min. The samples were subsequently poured into the columns under positive pressure (flow rate of ~2 mL/min), washed with 1 mL of 1.0 M acetic acid and dried with maximum pressure for at least 30 s; the inside of each column was wiped out with a fresh Kimwipe®. The columns were additionally washed with 3 mL of methanol, and again dried with maximum pressure for a minimum of 30 s with the tips wiped with Kimwipes. Labeled 12 × 75 mm test tubes were placed under the corresponding columns into which the analytes were eluted with 3 mL of methylene chloride : isopropanol : ammonium hydroxide (78 : 20 : 2, v/v/v) at a flow rate of ~2 mL/min; 30 μL of methanol with 1% hydrochloric acid was added afterward to each sample including the unextracted standard. The eluates were evaporated to dryness under nitrogen in the TurboVap® (Zymark Corporation, Hopkinton, MA, USA) at 40 ± 5°C, reconstituted with 100 μL of ethyl acetate, derivatized with 100 μL of PFPA and incubated on the heating block for 30 min at 65 ± 5°C. The halogenated derivatives were evaporated to dryness under nitrogen at 40 ± 5°C, reconstituted with 100 μL of ethyl acetate and transferred to autosampler vials.

Analytes were quantified employing a Hewlett-Packard 5890 GC interfaced with a Hewlett-Packard 5972 mass selective detector (MSD) and equipped with a Hewlett-Packard 7673 Automatic Liquid Sampler (Wilmington, DE, USA). The extract (1.0 μL, splitless) was injected onto the GC–MS system and analytical separation was achieved on an Rxi®-5Sil MS column (30 m × 0.25 mm i.d. × 0.25 μm; Restek, State College, PA, USA). Helium was the carrier gas flowing at 1.0 mL/min, and the inlet temperature of the GC was 250°C. The oven temperature was initially 80°C for 0.5 min, ramped 20°C/min to 320°C and held for 2 min. The analytes were detected by the MSD operated in selective ion mode, and the generated data were analyzed by the Hewlett-Packard ChemStation configured with the DrugQuant Software. Instrumental parameters including retention time, quantitative ion and qualifier ions are indicated in Table II.

The lower limit of quantification (LOQ) was 25 ng/mL, the limit of detection was 7 ng/mL and reportable range of linearity was 25–500 ng/mL for each analyte (ethylone, mephedrone, methylone, DOB, DOC and DOI). When the quantified concentrations exceeded 500 ng/mL, the specimen was re-analyzed with less specimen volume. Correlation coefficients for the five-point calibration curves were >0.99; all calibrators and positive control samples were quantified within 20% of the expected concentration. The intra-run imprecision was 6.6% CV, whereas the inter-run imprecision was 7.7% CV. The bias was 2.1%. The linearity, imprecision and bias values were calculated with the calibrators and positive control samples (at 100 ng/mL) in human drug-free whole blood fortified with the six analytes and analyzed over 5 days. Butylone, a structural isomer of ethylone, was chromatographically separated and verified not to interfere with the analysis of ethylone.

Table II
Instrumental Parameters Including Retention Time, Quantitative Ion and Qualifier Ions

Analyte	Retention time (min)	Quantitative ion (*m/z*)	Qualifier ions (*m/z*)
Ethylone	8.35	218	190, 367
Ethylone-d_5	8.34	223	372
Mephedrone	6.59	204	160, 205
Mephedrone-d_3	6.58	207	163
Methylone	8.01	204	160, 205
Methylone-d_3	8.00	207	163
DOB	8.50	256	258, 419
DOC	8.08	375	212, 214
DOI	8.99	277	304, 467
MBDB-d_5[a]	7.95	222	178

DOB, 4-bromo-2,5-dimethoxyamphetamine; DOC, 4-chloro-2,5-dimethoxyamphetamine; DOI, 4-iodo-2,5-dimethoxyamphetamine.
[a]Deuterated MBDB was utilized as an internal standard for the quantification of DOB, DOC and DOI.

Results

In all nine cases, ethylone was presumptively identified in urine or bile (Cases 4 and 6 where the quantity of the postmortem urine sample was not sufficient) via the full scan GC–MS drug screen. The presence of the drug was subsequently confirmed by the GC–MS in selective ion mode. Ethylone concentrations in seven postmortem blood samples ranged from 38 to 2,572 ng/mL; in one case, ethylone was identified but the concentration was below LOQ (25 ng/mL), and in the other case, the blood sample was negative for ethylone. No other synthetic amphetamines included in the assay (mephedrone, methylone, DOB, DOC or DOI) were detected in any blood samples. Besides ethylone, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) was detected in urine samples of all cases but one. In the seven cases, 1–7 additional drug(s), metabolite(s) and/or ethanol were identified; some of these compounds were confirmed and quantified in blood samples. Interestingly, in the five cases where ethylone concentration in blood was high (468–2,572 ng/mL), no drugs other than THCCOOH and/or ethanol were found in urine or blood except in one case where doxylamine was additionally identified. The results of the toxicological analyses are summarized in Table III.

Discussion

This report documented multiple postmortem cases with confirmed blood ethylone concentrations. Drug testing programs started to detect ethylone in the 'bath salt' products and urine samples in late 2011. Ethylone could be one of the drugs replacing more strictly controlled, earlier generation of synthetic cathinones like methylone, mephedrone and MDPV. In August 2014, the New York Customs and Border Protection confiscated an international package containing a large amount of ethylone sent to an individual who was later charged with possession of controlled substances with intent to distribute (17). Simmler *et al.* (10) demonstrated that ethylone was an equipotent inhibitor of all three dopamine, norepinephrine and serotonin transporters, and compared with its non-β-ketone analog (3,4-methylene dioxy-*N*-ethylamphetamine, MDEA), it was more selective for the dopamine system than the serotonin system. The researchers postulated that this propensity may be associated with a higher risk of addiction than MDEA, also a drug of abuse (10). There has not been a study examining the addiction potential of ethylone. However, nearly half of the 1,506 online survey respondents who reported using mephedrone at least once found it to be addictive (18), and the potency of ethylone for inhibiting dopamine reuptake was shown comparable with that of mephedrone (10).

Table III
Results of Toxicological Analyses

Case	Ethylone			Other compounds detected			
	Blood, ng/mL (source of blood)	Urine	Bile	Blood	Urine	Bile	Vitreous humor
1	<25 (inferior vena cava)	Positive	—	Alprazolam (29 ng/mL)	α-Hydroxyalprazolam, THCCOOH, cocaine, benzoylecgonine, levamisole, α-PVP	—	—
2	2,572 (right iliac vein)	Positive	—	Ethanol (0.044 g/dL)	Ethanol (0.072 g/dL), THCCOOH	—	Ethanol (0.060 g/dL)
3	ND (peripheral)	Positive	—	Alprazolam (82 ng/mL), benzoylecgonine (568 ng/mL), morphine (free) ND, morphine (total, 102 ng/mL)	α-Hydroxyalprazolam, THCCOOH, cocaine, benzoylecgonine, codeine, levamisole, morphine, 6-acetylmorphine	—	—
4	1,837 (left iliac vein)	QNS	Positive	—	THCCOOH	—	—
5	38 (peripheral)	Positive	—	Diphenhydramine[a], tramadol[a]	Acetone (185 mg/dL), THCCOOH, diphenhydramine, tramadol, *N*-desmethyltramadol	—	—
6	173 (heart)	QNS	Positive	Ethanol (0.029 g/dL)	QNS	Ethanol (0.034 g/dL)	Ethanol (0.060 g/dL)
7	1,617 (inferior vena cava)	Positive	—	—	Ethanol (0.033 g/dL), THCCOOH	—	—
8	1,725 (cavity fluid)	Positive	—	Ethanol (0.333 g/dL), doxylamine	Ethanol (0.245 g/dL), doxylamine	—	Ethanol (0.244 g/dL)
9	468 (peripheral)	Positive	—	Ethanol (0.128 g/dL)	Ethanol (0.151 g/dL), THCCOOH	—	—

AM, antemortem; PM, postmortem; QNS, quantity not sufficient; ND, not detected; α-PVP, alpha-pyrrolidinopentiophenone; THCCOOH, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol.
[a]Present at concentration less than the assay limit of quantitation (0.5 mg/L).

Synthetic cathinone users presented at medical facilities frequently reported psychological, cardiovascular and neurologic sympathomimetic symptoms including agitation, psychosis, aggression, tachycardia, palpitation, vasoconstriction, chest pain, seizure and headache (19–23). Similarly, the decedents who were positive for mephedrone and MDPV exhibited agitation, delusion, tachycardia, chest pain, hyperthermia and vomiting, prior to death (24–26). Self-inflicted injury and strange or risky behavior commonly contributed to the death of synthetic cathinone users, along with polydrug use (27–29). The San Diego County Medical Examiner's Office recently reported two multi-drug intoxication fatalities in which ethylone was quantified at 390 ng/mL (peripheral) and 380 ng/mL (central) in one case, and 40 ng/mL (central) for the other; additional drugs detected in blood/urine included opioids, alprazolam and THC among others (30). In the current study, we also found multiple other drugs including THCCOOH, cocaine, alprazolam and opioids, particularly in the cases where blood ethylone concentrations were <200 ng/mL. Other synthetic cathinone postmortem cases documented concurrent detection of THCCOOH, cocaine, antidepressants, amphetamines, opioids and benzodiazepines (24, 27, 31).

As synthetic cathinones are structurally similar to amphetamine and its derivatives, the drugs may produce positive results in assays targeting amphetamine/methamphetamine. Urine samples in three (Cases 2, 5 and 7) of the nine present cases gave a positive result in the CEDIA amphetamine/ecstasy assay, which had ≥100% cross-reactivity with amphetamine, methamphetamine, MDMA and their derivatives. Because ethylone blood concentrations in those cases varied widely and other cases with a high ethylone concentration (Cases 4 and 8) did not produce a positive result, contribution of ethylone to the positive results is difficult to conclude. It is possible that other substances not tested in our laboratory may solely or partially contribute to the positive results. In another study, oral fluid samples fortified with methedrone, methylone, mephedrone, MDPV, fluoromethcathinone, fluoromethamphetamine, 1-(3-chlorophenyl)piperazine (mCPP) and 3-trifluoromethylphenylpiperazine (TFMPP) at 10 and 100 μg/mL were tested positive for methamphetamine but not for amphetamine, using the Dräger DrugTest 5000 (32). When the cross-reactivity of 39 new amphetamine-type designer drugs was evaluated on Screen Multi-Drug and GIMA One Step Multi-Line Screen Test oral fluid devices, *p*-methoxyampheta mine (PMA) and *p*-methoxymethamphetamine (PMMA) gave positive results for amphetamine and methamphetamine, respectively, at 20–200 ng/mL (33). Swortwood *et al.* (34) tested 16 different ELISA reagents for the cross-reactivity of 30 designer drugs including 8 cathinone derivatives; only MDA, MDMA, ethylamphetamine and α-methyltryptamine showed significant cross-reactivities (30–250%). The findings highlighted the importance of more specific, chromatographic screens for the detection of synthetic cathinones including ethylone.

The growing use of synthetic cathinones in recent years is a critical public health concern given the paucity of research on their toxicity, addiction potential and withdrawal symptoms. Ethylone is a newer synthetic cathinone that our laboratory began to observe in decedents in 2014. Blood ethylone concentrations can exceed 1,000 ng/mL; on the other hand, the concentrations can be below 25 ng/mL, so sensitivity of the analytical method is essential. This report raises awareness in the forensic toxicology community of this novel synthetic cathinone.

References

1. Prosser, J.M., Nelson, L.S. (2012) The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology*, **8**, 33–42.
2. Gregg, R.A., Rawls, S.M. (2014) Behavioral pharmacology of designer cathinones: a review of the preclinical literature. *Life Sciences*, **97**, 27–30.
3. Valente, M.J., Guedes de Pinho, P., de Lourdes Bastos, M., Carvalho, F., Carvalho, M. (2014) Khat and synthetic cathinones: a review. *Archives of Toxicology*, **88**, 15–45.
4. American Association of Poison Control Centers. (2014) *Bath salts data 2011–2014*. https://aapcc.s3.amazonaws.com/files/library/Bath_Salts_Web_Data_through_7.2014.pdf (accessed Sep 15, 2014).
5. National Forensic Laboratory Information System. (2014) *Special report: synthetic cannabinoids and synthetic cathinones reported in nflis, 2010–2013*. https://www.nflis.deadiversion.usdoj.gov/Desk

topModules/ReportDownloads/Reports/NFLIS_SR_CathCan_508.pdf (accessed Sep 15, 2014).
6. Zawilska, J.B., Wojcieszak, J. (2013) Designer cathinones—an emerging class of novel recreational drugs. *Forensic Science International*, **231**, 42–53.
7. Drug Enforcement Administration. (2011) *Federal register vol. 76, no. 204*. www.gpo.gov/fdsys/pkg/FR-2011-10-21/pdf/2011-27282.pdf (accessed Sep 15, 2014).
8. Drug Enforcement Administration. (2014) *List of scheduling actions, controlled substances, regulated chemicals*. http://www.deadiversion.usdoj.gov/fed_regs/rules/2014/fr0307_2.htm (accessed Sep 15, 2014).
9. Schneir, A., Ly, B.T., Casagrande, K., Darracq, M., Offerman, S.R., Thornton, S. *et al.* (2014) Comprehensive analysis of 'bath salts' purchased from California stores and the internet. *Clinical Toxicology (Phila)*, **52**, 651–658.
10. Simmler, L.D., Buser, T.A., Donzelli, M., Schramm, Y., Dieu, L.H., Huwyler, J. *et al.* (2013) Pharmacological characterization of designer cathinones in vitro. *British Journal of Pharmacology*, **168**, 458–470.
11. Zaitsu, K., Katagi, M., Kamata, H.T., Kamata, T., Shima, N., Miki, A. *et al.* (2009) Determination of the metabolites of the new designer drugs bk-MBDB and bk-MDEA in human urine. *Forensic Science International*, **188**, 131–139.
12. Mueller, D.M., Rentsch, K.M. (2012) Generation of metabolites by an automated online metabolism method using human liver microsomes with subsequent identification by LC–MS(n), and metabolism of 11 cathinones. *Analytical and Bioanalytical Chemistry*, **402**, 2141–2151.
13. Zaitsu, K., Katagi, M., Tatsuno, M., Sato, T., Tsuchihashi, H., Tsuchihashi, K. (2011) Recently abused β-keto derivatives of 3,4-methylenedioxyphenylalkylamines: a review of their metabolisms and toxicological analysis. *Forensic Toxicology*, **29**, 73–84.
14. Uralets, V., Rana, S., Morgan, S., Ross, W. (2014) Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine. *Journal of Analytical Toxicology*, **38**, 233–241.
15. Rust, K.Y., Baumgartner, M.R., Dally, A.M., Kraemer, T. (2012) Prevalence of new psychoactive substances: a retrospective study in hair. *Drug Testing and Analysis*, **4**, 402–408.
16. Knoy, J.L., Peterson, B.L., Couper, F.J. (2014) Suspected impaired driving case involving α-pyrrolidinovalerophenone, methylone and ethylone. *Journal of Analytical Toxicology*, **38**, 615–617.
17. McGovern, P. (2014) *Weehawken man faces drugs, weapons charges after taking delivery of drugs: police*. The Jersey Journal. http://www.nj.com/hudson/index.ssf/2014/08/weehawken_man_caught_by_authorities_with_various_drugs_and_hollow_point_bullets_police.html#incart_related_stories (accessed Sep 15, 2014).
18. Carhart-Harris, R.L., King, L.A., Nutt, D.J. (2011) A web-based survey on mephedrone. *Drug and Alcohol Dependence*, **118**, 19–22.
19. James, D., Adams, R.D., Spears, R., Cooper, G., Lupton, D.J., Thompson, J.P. *et al.* (2011) Clinical characteristics of mephedrone toxicity reported to the UK National poisons information service. *Emergency Medicine Journal*, **28**, 686–689.
20. Regan, L., Mitchelson, M., Macdonald, C. (2011) Mephedrone toxicity in a Scottish emergency department. *Emergency Medicine Journal*, **28**, 1055–1058.
21. Centers for Disease Control and Prevention. (2011) Emergency department visits after use of a drug sold as 'bath salts'—Michigan, November 13, 2010-March 31, 2011. *Morbidity Mortality Weekly Report*, **60**, 624–627.
22. Wood, D.M., Greene, S.L., Dargan, P.I. (2011) Clinical pattern of toxicity associated with the novel synthetic cathinone mephedrone. *Emergency Medicine Journal*, **28**, 208–302.
23. Spiller, H.A., Ryan, M.L., Weston, R.G., Jansen, J. (2011) Clinical experience with and analytical confirmation of 'bath salts' and 'legal highs' (synthetic cathinones) in the United States. *Clinical Toxicology (Phila)*, **49**, 499–505.
24. Lusthof, K.J., Oosting, R., Maes, A., Verschraagen, M., Dijkhuizen, A., Sprong, A.G. (2011) A case of extreme agitation and death after the use of mephedrone in the Netherlands. *Forensic Science International*, **206**, e93–e95.
25. Maskell, P.D., De Paoli, G., Seneviratne, C., Pounder, D.J. (2011) Mephedrone (4-methylmethcathinone)-related deaths. *Journal of Analytical Toxicology*, **35**, 188–191.
26. Kesha, K., Boggs, C.L., Ripple, M.G., Allan, C.H., Levine, B., Jufer-Phipps, R. *et al.* (2013) Methylenedioxypyrovalerone ('bath salts'), related death: case report and review of the literature. *Journal of Forensic Science*, **58**, 1654–1659.
27. Marinetti, L.J., Antonides, H.M. (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. *Journal of Analytical Toxicology*, **37**, 135–146.
28. German, C.L., Fleckenstein, A.E., Hanson, G.R. (2014) Bath salts and synthetic cathinones: an emerging designer drug phenomenon. *Life Sciences*, **97**, 2–8.
29. Schifano, F., Corkery, J., Ghodse, A.H. (2012) Suspected and confirmed fatalities associated with mephedrone (4-methylmethcathinone, 'meow meow') in the United Kingdom. *Journal of Clinical Psychopharmacology*, **32**, 710–714.
30. McIntyre, I.M., Hamm, C.E., Sherrard, J.L., Gary, R.D., Burton, C.G., Mena, O. (2015) Acute 3,4-methylenedioxy-*N*-ethylcathinone (ethylone) intoxication and related fatality: a case report with postmortem concentrations. *Journal of Analytical Toxicology*, **39**, 225–228.
31. Torrance, H., Cooper, G. (2010) The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. *Forensic Science International*, **202**, e62–e63.
32. de Castro, A., Lendoiro, E., Fernández-Vega, H., Steinmeyer, S., López-Rivadulla, M., Cruz, A. (2014) Liquid chromatography tandem mass spectrometry determination of selected synthetic cathinones and two piperazines in oral fluid. Cross reactivity study with an on-site immunoassay device. *Journal of Chromatography A*, **1374**, 93–101.
33. Nieddu, M., Burrai, L., Trignano, C., Boatto, G. (2014) Evaluation of commercial multi-drug oral fluid devices to identify 39 new amphetamine-designer drugs. *Legal Medicine (Tokyo)*, **16**, 106–109.
34. Swortwood, M.J., Hearn, W.L., DeCaprio, A.P. (2014) Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays. *Drug Testing and Analysis*, **6**, 716–727.