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Case Report

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Reporting Two Fatalities Associated with the Use of 4-Methylethcathinone (4-MEC) and a Review of the Literature

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Abstract

We report two fatalities that are related to the cathinone 4-methylethcathinone (4-MEC) and review the current knowledge of 4-MEC. Qualitative and quantitative analysis of 4-MEC was performed by validated high performance liquid chromatography–tandem mass spectrometry methods. In the first case a 22-year-old male died in hospital following collapse and seizures after using 4-MEC. Toxicological analysis of postmortem femoral blood revealed the presence of 4-MEC (0.167 mg/L), ethanol (27 mg/100 mL) and paracetamol (5 mg/L). Death was attributed solely to 4-MEC toxicity. The second case involved a 54-year-old man found with a taped plastic bag over his head. Toxicological analysis of postmortem femoral blood revealed the presence of 4-MEC (1.73 mg/L) along with ethanol (229 mg/100 mL), propranolol (0.036 mg/L), venlafaxine (0.284 mg/L) and its metabolite O-desmethylvenlafaxine (0.205 mg/L), and diazepam (<0.005 mg/L) and its metabolite nordiazepam (0.033 mg/L). Death was attributed primarily to asphyxiation. These cases and a review of the current knowledge of 4-MEC pharmacology/toxicology adds to the body of case material for 4-MEC and will assist with interpretation in postmortem toxicology cases in which 4-MEC is detected.

Introduction

4-Methylethcathinone [4-MEC; 4-methyl-N-ethylcathinone; IUPAC: (R/S)-2-(ethylamino)-1-(4-methylphenyl)propan-1-one] (Figure 1A) is one of a number of synthetic derivatives of the pharmacologically active alkaloid cathinone (Figure 1B). Cathinone is the major pharmacologically active ingredient of the leaves of the evergreen khat plant (*Catha edulis*), a shrub native to Eastern Africa and the Arabian Peninsula used and abused for its stimulant effects (1). 4-MEC is considered to be amongst the first of the second wave of synthetic cathinones (including flephedrone, ethylone and 4-methyl-α-pyrrolidinopropiophenone (2, 3)) and was first detected around Europe in 2010 (4–8). 4-MEC was being marketed as an alternative to the first generation synthetic cathinones, such as mephedrone (9). There has been an increasing number of detections of 4-MEC in the UK (postmortem cases). Over 3 years (2010–2012) it was shown that 4-MEC was the second most common cathinone detected after mephedrone in the UK (10), This was also mirrored in Poland where there were increased detections in "legal high" preparations (4, 8). 4-MEC was controlled in the UK in April 2010 (11).

There is a paucity of information on the clinical effects of 4-MEC in humans due to its illicit nature as 4-MEC is not subject to the same development processes as licenced medicines. The clearest picture of the effects of 4-MEC comes from user experiences that are commonly published by the so-called "psychonauts" (urbandictionary.com definition

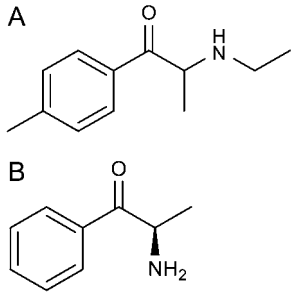

Figure 1. Structures of (A) 4-MEC (4-methylethcathinone) and (B) cathinone.

"a psychonaut – a person who intelligently experiments with mind-altering chemicals, sometimes to the extent of taking exact measurements and keeping records of experiences") on various "research chemical" websites (i.e., www.erowid.org, www.bluelight.ru and www.drugs-forum.com). A systematic review of these 4-MEC reports (23 "trip" reports and 112 "forum screenshots") was published by Van Hout (12). This research shows that the majority of 4-MEC users reporting are males between the ages of 30 and 45 years and have previous history of new psychoactive substance (NPS) use (12).

4-MEC has been reported to have been taken mainly by the oral or nasal route, but there are isolated reports of intravenous, ocular ("eyeballing") and rectal ("booty bumping" or "plugging") administration (12). The common dosages being 5–200 mg (nasal) and 15–300 mg (oral) with users reporting that doses of >75 mg (nasal) and >100 mg (oral) were required for the "extreme activity" of 4-MEC (12). The common "positive" effects of 4-MEC reported are "heightened consciousness, general mood lift, detached dissociative feeling, increased body temperature, sensual enhancement, waves of euphoria, deep relaxation and increased appreciation of music" (12). The negative effects of 4-MEC reported were head fogginess, migraine, loss of sight, heart palpitations, laxative effects, excessive sweeting, nausea, vomiting, jaw clenching, nystagmus and hiccups. Users did report that there was a low incidence of symptoms after cessation of positive effects unlike the agitation and insomnia reported with mephedrone (12, 13). This user data allows an insight into the effects of 4-MEC in an uncontrolled environment, however they may be limited by concomitant abuse of other drugs, and the possible lack of purity (or identity) of the product purchased as studies analyzing the chemical composition of purchased NPS have found varying purity and composition (4, 8). Two clinical cases of acute 4-MEC poisoning (with analytical confirmation) have been reported with symptoms. In the first case in the literature 4-MEC and gamma-butyrolactone (GBL) were injected and ingested respectively. The 39-year-old male was in a "confused" state and exhibited transient apnea (although this may be due to the GBL) (14), with normal sinus rhythm, heart rate of 80 bpm and "normal" blood pressure 120/80 mmHg (15). In a second case report, a 30-year-old male ingested a substance labeled "NRG3" and 4-MEC over 2 days, was admitted to hospital with "delirium" and tachycardia (heart rate not given) (16). The effects observed are consistent with the limited information that has emerged on the pharmacological and behavioral actions based on both *in vitro* and *in vivo* studies.

It is important in forensic case work (particularly in postmortem interpretation) to have a variety of cases (such as drugs deaths, driving under the influence of drugs, acute poisoning and cases with a non-drug related cause of death such as hanging) where a specific drug, in this case 4-MEC, was detected in order to help interpretation of the drug concentrations detected after death. In this paper, we report the details of two fatalities, one due to 4-MEC toxicity and the other due to asphyxia where 4-MEC was detected and quantitated in postmortem femoral venous blood by high-performance liquid chromatography tandem mass spectrometry. These cases add to the body of knowledge of 4-MEC and will help in the interpretation of cases where 4-MEC is detected and quantitated.

Case histories

Case 1

A 22-year-old male was believed to have insufflated an unknown quantity of an NPS along with consumption of alcohol and use of cannabis. A short time later he suddenly collapsed, started to convulse and coughed up blood. The emergency services were called and resuscitation was attempted at the scene. He was transported to hospital, and after further attempts to save his life, was pronounced dead ~4.5 hours after taking the NPS. Following a complete autopsy and appropriate ancillary investigations, no natural disease or injury which could have caused, contributed to or accelerated death were identified.

Case 2

A 54-year-old male disappeared from his home and was reported as a missing person. After a police search based on mobile phone records, the deceased was found in a wooded area with a plastic carrier bag taped over his head. The postmortem findings were consistent with asphyxia due to respiratory obstruction. There was also significant left ventricular hypertrophy and coronary atheroma.

Experimental

Initial toxicological screening

Unpreserved postmortem urine and postmortem blood samples were collected in 20 mL universal vials (for drug analysis). For alcohol analysis, postmortem samples were preserved with 2% sodium fluoride/potassium oxalate. All preserved blood and urine samples were analyzed for the presence of volatiles (ethanol, methanol, isopropanol and acetone) using an Agilent head space gas chromatography flame ionization detection system (Agilent 7820 A GC with Agilent G1888 headspace). Unpreserved urine samples were screened for drugs by gas chromatography–mass spectrometry Agilent 6890GC with 5,975 Mass Selective Detector using solid phase extraction (SPE) Telos NM pH 9 urine extraction tubes (Kinesis, UK) with drug matching to a commercial library (NIST05 and SWGDRUG). Unpreserved blood samples were screened using liquid chromatography with tandem mass spectrometry (LC–MS-MS) Agilent 6410B triple quadrupole with 1200 series high performance liquid chromatography (HPLC) system using SPE (Bond Elute Certify SPE; Agilent, UK) with drug matching to an in-house library constructed from certified reference standards. Screening methodology was adapted from common toxicology screening (17). Drugs detected during screening (from both urine and blood) were quantitated in unpreserved postmortem blood by LC–MS-MS using validated methods. Blood paracetamol concentrations were measured using an Agilent 1100 series HPLC system with diode array detection again using a validated method.

Quantitation of 4-MEC

Reagents and materials

Certified standards for 4-MEC and d3-mephedrone were purchased from LGC Standards (Teddington, UK). Methanol, chloroform, isopropanol, hydrochloric acid, 35% ammonia solution and glacial acetic acid were purchased from Fisher Scientific (Loughborough, UK). Potassium dihydrogen orthophosphate was purchased from BDH (Poole, UK). Strata Screen-C SPE cartridges were purchased from Phenomenex (Macclesfield, UK). Blank equine serum was purchased from TCS Biosciences (Buckingham, UK).

Standards, calibrators, control and internal standard preparation

A stock solution of 4-MEC was prepared at a concentration of 1 mg/mL in methanol and stored at −20°C. A calibration curve between 0.125 and 1 mg/L was prepared in blank equine serum. The internal standard (d3-mephedrone) was prepared at a concentration of 2 mg/L in methanol and stored at −20°C. Calibrator materials were prepared prior to each extraction. If required, postmortem blood samples were diluted in blank equine serum to fit into the calibration range. In-house quality control material was prepared from a separate stock solution of 4-MEC at levels of 0.150 and 0.750 mg/L in blank equine serum.

Extraction method for biological specimens

The extraction method was based on a standard SPE procedure (18). Briefly, 1 mL of the standard/control/biological sample was added to a 10 mL glass tube and spiked with 200 µL of internal standard (d3-mephedrone). 1 mL of phosphate buffer (0.1 M potassium dihydrogen orthophosphate in deionised water, pH 6.0) followed by 2 mL of deionised water was added to all tubes. Tubes were briefly vortexed and then sonicated for 10 min. After centrifugation at 3,500 rpm for 8 min, the supernatant was added to Phenomenex Strata Screen-C SPE columns (pre-conditioned with methanol, deionised water and 0.1 M phosphate buffer) and eluted at low speed (1–2 mL/min). After washing with deionised water, 0.1 M HCl and methanol, samples were eluted at low speed (1–2 mL/min) with 3 mL of elution buffer (800 mL chloroform, 200 mL isopropanol, 3 mL 35% ammonia solution) into 10 mL glass tubes. Samples were concentrated using a Techne-Dri Block (Techne, Staffordshire, UK) at 40°C under nitrogen and reconstituted in 1 mL of 10% methanol.

Instrumentation and LC–MS-MS parameters

Quantitative analysis of 4-MEC was performed using an Agilent Technologies 1200 Series HPLC system coupled with an Agilent Technologies 6460 Triple Quadrupole mass spectrometer. Agilent MassHunter Workstation Software B.03.01 was used for data analysis. Separation was carried out on an Agilent Zorbax SB-C18 30 mm × 2.1 mm column (particle size: 3.5 µm). A gradient elution program was applied at a flow rate of 0.6 mL/min with eluent A consisting of 0.1% glacial acetic acid in deionised water and eluent B consisting of 0.1% glacial acetic acid in methanol. Gradient elution was 0–1.5 min 10 → 100% B; 1.5–2.8 min 100% B; 2.8–4 min 100 → 10% B total run time of 4 min. Column temperature = 30°C, injection volume = 2 µL. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode using positive electrospray ionization. Drying gas (nitrogen) temperature was set to 350°C, gas flow rate to 5 L/min, nebulizer pressure to 45 psi, capillary voltage to 3,500 V, collision energy to 20 V; dwell time to 50 ms; V_{fr} was set to 100 V for 4-MEC and 90 V for the internal standard (d3-mephedrone). The following ion transitions and retention times were observed: 4-MEC (*m/z* 192/159 and 192/145), 0.86 min and the internal standard d3-mephedrone (*m/z* 181/148 and 181/122), 0.72 min (quantitation ions underlined).

Method validation and matrix effects

The LC–MS-MS method for 4-MEC quantitation was validated in serum using the conditions previously described and according to the guidelines of Peters *et al.* (19). Standard calibration curves for 4-MEC in equine serum were linear from 0.125 to 1 mg/L with an $r^2 > 0.999$. Using quality control samples of 0.150 mg/L and 0.750 mg/L the method was shown to be both accurate and precise (interday ($n = 5$) and intraday ($n = 30$) within acceptable ranges of ±15%). The limit of detection was found to be 0.002 mg/L (based on a signal:noise ratio of 3:1) and the limit of quantitation was 0.125 mg/L using the lowest calibration standard (CV 8%). The matrix effects were evaluated by the methods of Matuszewski *et al.* (20) at both 0.150 mg/L and 0.750 mg/L and although ion suppression/enhancement was observed results were within the accepted ±15%. Possible interference from isobaric compounds such as pentedrone were excluded by differentiation of retention times, fragmentation ions (pentedrone, *m/z* 192/132 and 192/91) and qualifier ion ratios. For positive identification of the unknown a relative retention time of ±5% of the reference material with MRM transitions ratios of the unknown being within ±20% of MRM transition ratios (within the same run) were required. The results from matrix matching experiments carried out between equine serum and human blood showed a good correlation between the matrices used, allowing equine serum to be used for the calibration curves.

Results

Case 1

Toxicological analysis of unpreserved postmortem femoral venous blood revealed the presence of ethanol (30 mg/100 mL), 4-MEC (0.170 mg/L) and paracetamol (5 mg/L). Ethanol (27 mg/100 ml), 4-MEC and paracetamol were also detected in urine, but not quantitated.

Case 2

Toxicological analysis of unpreserved postmortem femoral venous blood revealed the presence of 4-MEC (1.730 mg/L) along with ethanol (229 mg/100 mL), propranolol (0.036 mg/L), venlafaxine (0.284 mg/L) and its metabolite O-desmethylvenlafaxine (0.205 mg/L, diazepam (<0.005 mg/L) and its metabolite nordiazepam (0.033 mg/L). 4-MEC, ethanol, venlafaxine and O-desmethylvenlafaxine were detected in urine, but not quantitated. The deceased had been prescribed propranolol and venlafaxine.

Discussion

In this study we have reported the pathological findings and toxicology of two fatal cases in which 4-MEC was detected. They are not the first cases to be reported but add to the body of data which is emerging on 4-MEC. Table I lists the publications that have cited the concentration of 4-MEC in a variety of case types. The blood concentrations of 4-MEC that have previously been observed

Table I. Summary of 4-MEC concentrations in a variety of matrices and case types

Author	Case type	Cause of death	Concentration of 4-MEC (mg/L)	Other drug(s) detected
Gill *et al.* (4)	Not drug related	Road traffic accident	Blood (0.152 mg/L)	Blood ethanol (120 mg/L)
Elliott and Evans (10)	Not drug related	Not given	Blood (0.28 mg/L)	Not given
Elliott and Evans (10)	Not drug related	Not given	Blood (5.03 mg/L)	Not given
This study	Not drug related	Asphyxia	Femoral blood (1.730 mg/L)	Propranolol (0.036 mg/L), Venlafaxine (0.284 mg/L), O-Desmethylvenlafaxine (0.205 mg/L)
Gill *et al.* (4)	Drug death	PMA intoxication	Blood (0.056 mg/L)	PMA (2.347 mg/L), PMMA (0.030 mg/L) Amphetamine (0.378 mg/L), THC (1.3 ng/L) THCCOOH (54.7 ng/mL)
Rojek *et al.* (22)	Drug death	4-MEC intoxication	Femoral blood (1.200 mg/L)	Amphetamine (0.230 mg/L)
Bottinelli *et al.* (23)	Drug death	4-MEC intoxication	Peripheral blood (14.600 mg/L)	None
Elliott and Evans (10)	Drug death	"Drugs Death"	Femoral blood (5.83 mg/L)	Methylone (4.31 mg/L), Ethanol (45 mg/dL) Cannabinoids, Cocaine (low levels) Quinine (low levels), Hydroxyzine (low levels) 4-FMC (low levels)
Elliott and Evans (10)	Drug death	"Drugs Death"	Blood (0.46 mg/L)	Not reported
Elliott and Evans (10)	Drug death	"Drugs Death"	Blood (2.50 mg/L)	Not reported
Elliott and Evans (10)	Drug death	"Drugs Death"	Blood (17.3 mg/L)	Not reported
This study	Drug death	4-MEC intoxication	Femoral blood (0.170 mg/L)	Paracetamol (5 mg/L), Ethanol (30 mg/100 mL)
Gill *et al.* (4)	Clinical	Arrested for drug possession	Blood (0.046 mg/L)	None
Turcant *et al.* (15)	Clinical	Samples taken 8 hours post drug use	Plasma (0.353 mg/L) Urine (100 mg/L)	GBL (Plasma) 300 mg/L GBL (Urine) 1,000 mg/L
Elliott and Evans (10)	Clinical	Not reported	Blood (0.09 mg/L)	Not reported
Elliott and Evans (10)	Clinical	Not reported	Blood (0.20 mg/L)	Not reported
Knapp *et al.* (16)	Clinical	Samples taken ~2 days after cessation of drug taking (10 g 4-MEC, 5 g NRG3 consumed over 3 days)	Urine (0.2 mg/L) Hair (30 ng/mg)	Plasma - paracetamol (7,000 mg/mL) - tramadol (0.465 mg/L) Urine - MDMA (0.02 mg/L) - MDPV (0.02 mg/L) Hair - MDMA (2 ng/mg) - MDA (0.1 ng/mg) - MDPV (1 ng/mg) - Mephedrone (0.1 ng/mg) - Cocaine (1.7 ng/mg) - Tramadol (3.5 ng/mg) - Hydroxyzine (0.14 ng/mg) - Aripiprazole (11 ng/mg) - Haloperidol (0.01 ng/mg)

in "alternative causes of death" (that is cases where a cause of death not related to the toxicology was reported such as asphyxia) are between 0.152 and 5.03 mg/L (n = 3), the cases where 4-MEC has been associated with "drug death" (in the literature it is unclear if the cause of death is due directly to 4-MEC or potentially fatal drug interactions due to the presence of multiple other drugs) are between 0.46 and 14.6 mg/L (n = 6) and in "clinical" cases 0.046–0.2 mg/L; (n = 3; one case with 0.353 mg/L in plasma has been separated out as the blood: plasma ratio for 4-MEC is currently unknown). It is unfortunate that in some of the cases the sampling site is not reported, as it is well known that femoral blood, although not perfect, is thought to produce the most reliable results for postmortem interpretation as it is the blood site that suffers the least from postmortem changes in drug concentration (21). These 4-MEC case studies show a significant overlap between concentrations of 4-MEC in drug related deaths and deaths not thought to be related to drugs adding to the complication of case interpretation.

In our data (Case 2), in which a 4-MEC femoral blood concentration of 1.730 mg/L was detected, the deceased was found suffocated with the primary cause of death identified as asphyxia. The pathologist ascertained that coronary atherosclerosis or left ventricular hypertrophy had contributed to death. Although alcohol ingestion and the blood concentration of 4-MEC may have affected cognition in this case, it did not contribute to the actual cause of death. In contrast the femoral blood concentration of 4-MEC in Case 1 was much lower than Case 2 at 0.167 mg/L. This is within the levels seen in cases where drugs were not involved in the death (see Table I) and is to date the lowest reported blood concentration of 4-MEC where the cause of death was ascribed to 4-MEC based on the absence of an alternative cause of death. This could be due to various factors:- (i) in common with all stimulants and many other drugs, tolerance may develop to both the stimulant and euphoric effects of 4-MEC (24) and therefore there is considerable overlap between the nontoxic and toxic blood drug levels. Therefore, death from 4-MEC use may not be directly related to its prevailing blood concentration and may not be dose related; (ii) in Case 1 it is reported that associates of the deceased also consumed alcohol and insufflated 4-MEC with no significant adverse effects. This may suggest the role of other as yet unknown factors in susceptibility to toxicological effects of 4-MEC and related synthetic cathinones; (iii) there may have been further metabolism of 4-MEC in Case 1 whilst the deceased was alive and (iv) cathinones, including 4-MEC are also known to be particularly unstable in biological fluids (blood, plasma, urine) being influenced by both pH and temperature. Cathinones have been shown to be stable below pH 5.5 over 7 days and increasingly unstable at more alkaline pH (over pH 5.5) (25, 26). In a stability study at room temperature there was complete loss of 4-MEC in blood after 14 days (37 days in plasma) with a 50% loss of 4-MEC observed in ~2 days (blood) and 7 days (plasma), with at least one of the breakdown products formed being dihydro-4-MEC (also a metabolite of 4-MEC; Figure 2) (27). Although to the best of the authors knowledge no studies of stability have been carried out with 4-MEC at fridge (−4°C) or freezer (−20°C) temperatures. Based on mephedrone data that has a similar degradation profile at room temperature, 4-MEC will be stable in the freezer for 14 days (at least) and only around 4–5 days in the fridge for whole blood, plasma and urine (26, 28).

It is however possible in the postmortem environment (both in the body and after sampling) that 4-MEC (and other cathinones) may remain stable as in both humans and animals the pH of blood after death has been shown to decrease to below pH 5.5 after 24–48 hours (29, 30), the pH at which the cathinones have been found to be stable. This "stability" due to pH may, however, be offset by the decreased stability that may result from normal body temperature changes. Comparison of the stability of mephedrone in spiked blood (28) and spiked postmortem blood (26) suggests that mephedrone is stable for longer in postmortem blood. Unfortunately the pH of the blood was not taken in order to study the influence of pH on stability in postmortem blood samples. Further studies investigating 4-MEC would need to be carried out to determine the exact rate of degradation due to temperature and pH. This degradation of cathinones could actually lead to an underreporting of the levels of 4-MEC unless the samples are frozen or analyzed on receipt in the laboratory. In all of the cases thus far reported it is unclear how the instability of 4-MEC may have affected the reported results. It is important to note as with all forensic toxicology, analytical results need to be interpreted in the context of deceased's background, postmortem findings and any other investigations or relevant information.

With postmortem cases, knowledge of the pharmacokinetics and metabolism of parent drug and any pharmacological activity of the metabolites can result in a more comprehensive interpretation of cases where 4-MEC is detected in samples. The pharmacokinetics of 4-MEC are however unclear with no animal or human studies published in the literature to date. The best indications come from *in vivo* animal studies investigating locomotive activity and human case reports. In the human use reports, the onset of effects of 4-MEC took ~5 min (insufflation) and 15–45 min (oral) to occur and the effects lasted for 2–5 hours (24, 31). In a rat study (investigating locomotor stimulus) the stimulant effects of 4-MEC occurred within 30 min and lasted for around 2 hours (32). Further detailed pharmacokinetic studies, both human and animal would allow more detailed pharmacokinetic parameters to be determined. The Phase I metabolism of 4-MEC has been studied in both human liver microsomes (33) and pooled human urine (3, 18, 28, 33). The studies show that the metabolism of 4-MEC is similar to mephedrone (34) (see Figure 2). The major metabolites appear to be dihydro-4-MEC (also thought to be a degradation product of 4-MEC), nor-4-MEC and nor-dihydro-4-MEC with hydroxytolyl-4-MEC being a minor metabolite (18, 28), although due to a lack of reference standards reliable data in both blood and urine for metabolite concentrations is as yet unavailable. Based on the similarity of the metabolism to mephedrone it is likely that the major Phase I metabolic enzyme is cytochrome P450 2D6 (CYP2D6) (35). As with metabolites of mephedrone it is unclear whether the metabolites of 4-MEC have any pharmacological activity.

The general pharmacology of cathinones on the surface appear to be relatively simple with the cathinones that have been investigated so far acting to exert their effects via one or more monoamine transporters causing an increase in one (or more) of the monoamines (noradrenaline (NA), dopamine (DA) or serotonin (5-HT)) at nerve terminals (2, 36–38). There is however more complexity in the pharmacology of the cathinones with an understanding of the exact mechanism of action of the individual cathinones being important to understand their actions and any potential toxicity (2). The cathinones, along with other drugs of abuse such as cocaine, amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) can either act as a substrate (causing the relevant monoamine release) or a blocker (stopping reuptake of the monoamine from the synaptic terminal) at one or more of the transporters (39, 40). In some rare cases a compound (i.e., ethcathinone) can act as a "hybrid" exhibiting both substrate and blocker effects at various monoamine

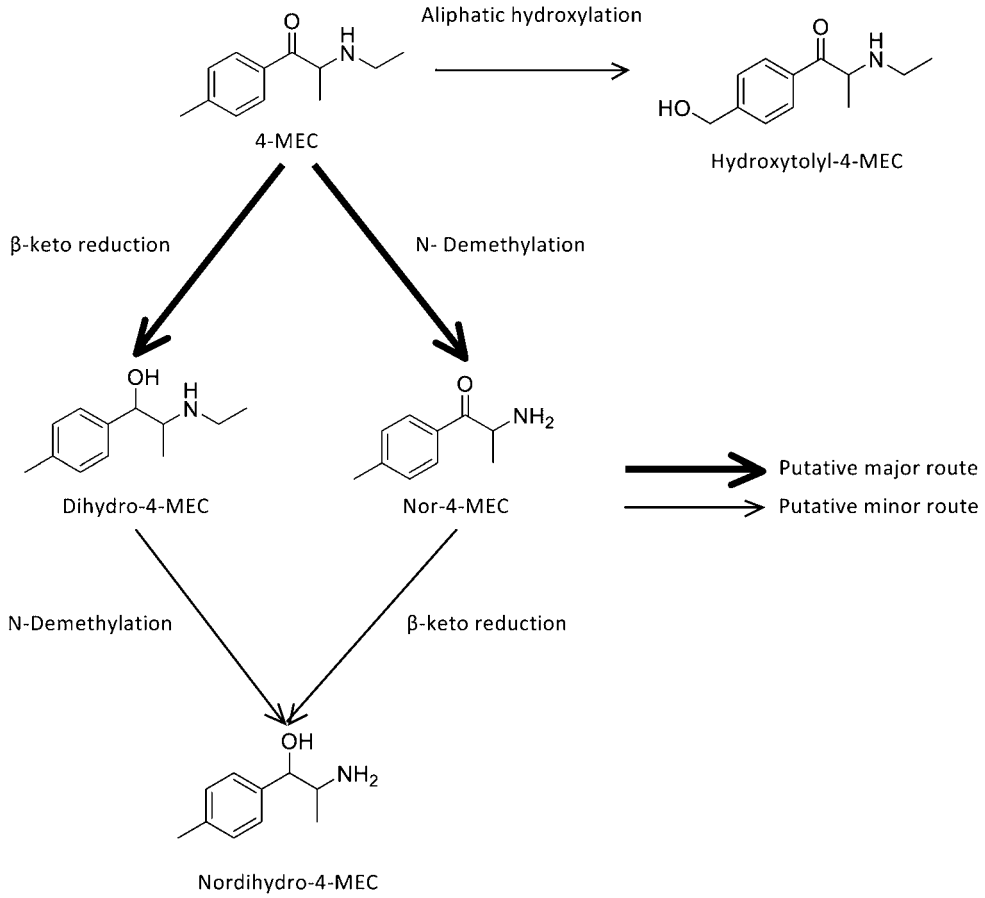

Figure 2. Phase I metabolic pathway of 4-MEC.

transporters (2, 32, 41). In studies using both *in vitro* and *in vivo* methodology 4-MEC have been found to exhibit the rare "hybrid" transporter activity acting as a substrate at 5-HT transporters causing 5-HT release and acting as a blocker at the DA, 5-HT and NA transporter at micromolar levels inhibiting DA, NA and 5-HT reuptake (2, 32, 42). The interaction of 4-MEC at a variety of receptors has been studied on human embryonic kidney 293 (HEK293) expressing cloned human monoamine transporters (32). 4-MEC has been shown to have micromolar affinity for 5-hydroxytriptamine-2A (5-HT_{2A}) and 5-HT_{2C} receptors (32) and sigma-2 receptors (σ2) (42) but no pharmacologically relevant affinity for other 5-HT receptor subtypes, DA, glutamate, gamma amino butyric acid, adrenoceptors, muscarinic or histamine receptors (32, 42).

With new drugs of abuse it is important to understand any long-term abuse potential. In human case reports there was an urge to re-dose whilst under the influence of 4-MEC but this appeared to significantly reduce after 24 h with the urge to re-dose thought to be due to the "weak, short lived effects" (12). This possible abuse potential of 4-MEC has been investigated with various *in vivo* animal behavioral models that are commonly used to assess the abuse liability of potential psychostimulants (such as locomotor stimulation, drug discrimination and self-stimulation) (43). 4-MEC was found to stimulate locomotor activity in mice in a dose-dependent manner similar to mephedrone and MDPV (43) although at a lower potency (31). In discrimination studies where the experimental model investigates the ability of the animal to respond to a novel drug when compared to known abused drugs (such as cocaine or methamphetamine) 4-MEC was found to fully substitute for the discriminative stimulus effects of methamphetamine and cocaine (31). The final behavioral study for 4-MEC that has been carried out to date is the intracranial self-stimulation (ICSS) paradigm that is thought to measure the activation of the brain reward pathway with reductions in ICSS thresholds indicating activation and increases in thresholds indicating inhibition of the reward pathway (33). 4-MEC was found to cause a decrease in ICSS threshold similar to those observed with methamphetamine (although at a 100-fold higher dose) indicating low potency (34).

The abuse liability of stimulant drugs is thought to increase with higher synaptic cleft concentrations of DA compared to 5-HT. This is expressed as DAT/SERT ratio and is commonly calculated by comparing the ability of the specific drug to inhibit the reuptake of DA/5-HT at the respective transporter (32, 35, 44). Experimental studies have shown that 4-MEC has a DAT/SERT ratio of ~1.85 (32). This is lower than the DAT/SERT ratio of methamphetamine (>10) (45) a drug with known significant abuse (46) but higher than that of MDMA (~0.08) (45). Although as 4-MEC also causes release of 5-HT (32) the abuse potential of 4-MEC is likely to be reduced. The hypothesis of greater concentrations of 5-HT rather than DA is backed up by *in vivo* rat micro dialysis studies of 4-MEC where higher concentrations of 5-HT than DA were measured in the nucleus accumbens (2). Overall these results suggest an abuse potential but lower than that of drugs such as cocaine and methamphetamine (31, 34). However, as with all drugs of abuse there are concerns over the long-term health dangers of chronic use.

Due to the relatively short time that 4-MEC has been abused, there is little data available. However, some predictions can be made based on the pharmacology of 4-MEC, from the structural similarity to MDMA and *in vitro* testing. In *in vitro* toxicity testing using rat hepatocytes 4-MEC was found to cause concentration dependent death of hepatocytes, however with a significantly lower potency when compared to MDMA (47) and also at a drug concentration ($EC_{50} = 1.29$ mM ≡ 246 mg/L) significantly higher than that observed in blood in a range of cases (see Table I). It is however common for liver drug concentrations to be higher than blood (14). To date no liver concentrations of 4-MEC have been published in the literature so it is not possible to comment further. Also, unlike MDMA, is unlikely that 4-MEC will cause valvular heart disease as all drugs that induce valvular heart disease in humans have been found to activate the 5-HT_{2B} receptor (48), a receptor that 4-MEC has been shown to have little or no affinity for (32, 42). Neurotoxicity studies (reviewed by Green *et al.* (49)) have indicated that it is unlikely that mephedrone would cause neurotoxicity, unless it is in the presence of other neurotoxic drugs (such as MDMA or amphetamine) (49). Due to a similar structure and reduced potency of 4-MEC (compared to mephedrone) it is likely to have a similar profile of neurotoxicity as mephedrone, specific studies on 4-MEC would however be able to clarify the neurotoxicity of 4-MEC.

Conclusion

This study has reported two cases to add to the current literature of postmortem 4-MEC blood concentrations, the first due to 4-MEC toxicity and the second highlighting a death unrelated to the toxicity of the drug. As with all "designer" cathinones it is recommend that in 4-MEC cases, or any case in which the presence of 4-MEC is suspected, the sample is stored at −20°C upon receipt and that analysis is carried out as soon as possible after thawing. The results and discussion of 4-MEC in this paper should further assist with the interpretation of any future 4-MEC cases.

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