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Fatal intoxication involving 4-methylpentedrone (4-MPD) in a context of chemsex

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Abstract

4-methylpentedrone (4-MPD) is a new psychoactive substance (NPS) belonging to the cathinone class. We report an original case of death mainly involving 4-MPD, along with cocaine, sildenafil, bromazepam and nevirapine. The investigation data and the autopsy findings indicated fatal intoxication in a chemsex context (drug use during sex). 4-MPD concentrations were determined in peripheral blood (1,285 ng/mL), cardiac blood (1,128 ng/mL), urine (>10,000 ng/mL), bile (1,187 ng/mL) and vitreous humor (734 and 875 ng/mL in left and right samples, respectively) by gas chromatography (GC) coupled to tandem mass spectrometry. 4-MPD metabolites were explored by GC coupled to high resolution mass spectrometry. Due to the paucity of data concerning 4-MPD, its use and effects were gathered from online user testimonies. This case illustrates the toxicity of this infrequent pentedrone derivate and confirms the significant overdose risk associated with chemsex.

Keywords

Forensic Toxicology – New Psychoactive Substance (NPS) - Fatal intoxication - 4-Methylpentedrone (4-MPD) – Chemsex

Highlights

4-methylpentedrone (4-MPD) is an infrequent cathinone that can be used for sexual purposes (chemsex).
A fatal intoxication mainly involving 4-MPD and cocaine was reported.
Postmortem 4-MPD distribution in biological fluids is described and its metabolism explored.
Online user testimonies were reviewed to understand the profile of this new psychoactive substance (NPS).
This case report illustrates the significant toxicity of 4-MPD and the overdose risk associated with chemsex

Introduction

New psychoactive substances (NPS) are a growing field of experimentation for drug users around the world. Since the 2000s, hundreds of these substances have appeared and spread, mainly on the internet market. Synthetic cathinones are one of the most active groups (1). Beside the stimulant effect common to all synthetic cathinones, the wide range of structural variation induces various public and health risk profiles and constitutes a challenge for legislation, identification and control (2,3). Collecting reliable data on these molecules is obviously of clinical interest to help in documenting their toxicity and to improve the patient care in case of intoxication. Synthetic cathinones, alongside other NPSs, are new additions to the traditional ‘club drugs’ such as ecstasy and cocaine for their stimulant and entactogenic effect (4). Cathinones can also be used in preparation for or during sexual intercourse or sex parties, especially among men having sex with men. This specific pattern of drug use, called “chemsex”, consists in engaging in sexual activities while under the influence of chemicals (4). This practice has been reported in both the USA and the UK, and is becoming common in other European countries (5). The intended effects of drug consumption include empathy, euphoria, increased energy and confidence,

lowered inhibition, enhanced sexual desire and pleasure, facilitated anal intercourse and improved sexual performance. Drugs can be taken orally, by inhalation, insufflation, injected or even administered by rectal route. The most popular drugs used in this context are crystal methamphetamine, gamma-hydroxybutyric acid (GHB) or gamma-butyrolactone (GBL), mephedrone and, to a lesser extent, cocaine and ketamine (6). Since the prohibition of mephedrone, for example in 2010 in France and United-Kingdom, the chemsex chemical arsenal has largely consisted of new cathinones (7). In sex parties, according to user reports, cathinones trigger feelings of sexual arousal, increase sexual longevity and facilitate having a greater number of sexual partners (4). Chemsex causes many health concerns: on the one hand, human immunodeficiency virus (HIV) and hepatitis virus (HVC) transmission due to high risk behavior (8); on the other hand, physical and mental drug side-effects, which can have dramatic consequences (9), often underestimated by users who do not consider themselves addicted and are less prone to seek proper information on the substances and professional help (6,7).

We report a case of 4-methylpentedrone (4-MPD, 4-methyl- α -methylamino-valerophenone) and cocaine intoxication with fatal outcome in a context of chemsex. 4-MPD is a stimulant drug of the cathinone class derived from pentedrone. It was firstly notified to European and French early warning systems in 2014 (Czech Republic) and 2017, respectively (10,11). To the best of our knowledge, this case is the first death involving 4-MPD. Our report focuses on autopsy findings, postmortem distribution in biological fluids and metabolism. As no reliable scientific data were available for 4-MPD, its use and toxicity were explored through a review of user testimonies available on the Internet.

Case report

A 57-year-old white male was found dead in a hotel room by the manager after he missed checkout. He was lying face down on the bed, naked, with an open bottle of alkyl nitrites (“poppers”) in the left hand. The early investigation in the room revealed an unidentified bag of white powder and the presence of sex-toys. The inquiry established that the victim was homosexual and had rented the place for the day for a sexual encounter. Relatives reported that the victim was HIV-positive, under treatment, and had a history of popper abuse. The medical examiner’s on-scene examination found no signs of violence.

Autopsy findings

A forensic autopsy was performed the day following the discovery of the corpse. The body showed significant external evidence of hypoxia syndrome, with severe cyanosis of the face, especially intense on the lips and ears, and of the upper part of the body (collar cyanosis) along with subungual cyanosis of the hands. Both eyes were affected with conjunctival hyperemia. No petechiae or puncture wounds were found on external body examination. All organs had a congested appearance. The lungs presented a few petechiae on the posterior face and mild edema. The heart was subnormal, with a weight of 420 g (body weight = 58 kg, height = 164 cm) (12) and a slightly dilated left ventricular cavity. The liver showed mild steatosis and had a variegated aspect congruent with congestive hepatopathy. The thymus gland was still present despite the subject’s age. The rest of the internal examination was unremarkable. No pills were found in the stomach. Samples of femoral venous and cardiac blood, bile, urine, lateralized vitreous humor (VH) and nasal swabs were collected and stored at +4°C until analysis.

Toxicological analyses

Standards and reagents

Certified stock solution of mephedrone-d3 at a concentration of 0.1 mg/mL was purchased from Euromedex (Souffelweyersheim, France). 4-MPD powder, purchased from LGC Standards (Molsheim, France), was used for the preparation of appropriate dilutions in methanol for calibration standards and quality control (QC). Drug-Screen® immunochromatographic assays were purchased from Nal Von Minden (Regensburg, Germany). Strata X Drug B solid-phase extraction (SPE) cartridges (3 mL, 60 mg) were purchased from Phenomenex (Le Pecq, France). Beta-glucuronidase from type VII-A Escherichia coli was obtained from Sigma Aldrich (Saint Quentin Fallavier, France).

Standard toxicological analyses

Urine immunological screening for drugs of abuse was performed. General unknown screening (GUS) was conducted by gas chromatography (GC) coupled to mass spectrometry (MS) (7890B, 5977A, Agilent Technologies, Les Ulis, France) with and without acetylation step, and by liquid chromatography with diode-array detector associated to mass detection (LC/DAD-MS, 1260 infinity, 6120 quadrupole LC/MS, Agilent Technologies). Ethanol was analyzed by headspace gas chromatography (HS-GC) with flame ionization detector (Agilent Technologies). Alkyl nitrites were investigated by HS-GC/MS. Drugs of abuse belonging to opioid, cannabinoid, amphetaminic and cocaine families were screened for by GC/MS. Nasal swabs were extracted by sonication in chloroform/toluene (50:50, v/v) and analyzed, after acetylation, for GUS by GC/MS.

Quantification of 4-MPD

The extraction process was adapted from a previously described method (13). Briefly, blood, urine, VH and bile samples (1 mL) were spiked with mephedrone-d3 at final concentration of 200 ng/mL. 2 mL sodium acetate buffer (100 mM, pH = 5) and 2 mL methanol/acetonitrile (85:15 v/v) were added. After agitation and centrifugation, the supernatant was purified by SPE. Two washing steps were performed (1 mL HCl 0.1 M; 1 mL acetonitrile/HCl 0.1M (70:30 v/v)) prior to elution by 2 mL ethyl acetate/isopropanol/NH₄OH (70:20:10 v/v/v). 200 µL methanol/acetic acid (98:2 v/v) solution was added before evaporation to dryness under nitrogen stream at 50°C. Dry residues were acetylated (60 µL acetic anhydride + 40 µL pyridine, 2 min at microwave 800W), evaporated and reconstituted in 40µL ethyl acetate. One microliter of sample was injected in splitless mode in the GC/MS-MS system (7890A GC, 7000 Triple Quadrupole GC/MS detector, Agilent Technologies). Separation was performed on an HP-5MS capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness; Agilent Technologies). The initial oven temperature of 90°C was held for 1 min, increased by 25°C/min to 200°C, then by 15°C/min to 300°C and held for 2 min at 300°C. Helium was used as carrier gas at a constant flow of 1.2 mL/min. The injector and the transfer line were set at 260°C and 320°C respectively. Multiple reaction monitoring (MRM) was used for 4-MPD (quantifier: 247.2-128.1; qualifiers: 247.2-86.1; 218.2-118.8; retention time (RT) 10.02 min) and mephedrone-d3 (quantifier: 103 – 61; qualifier: 222-103; RT 9.19 min).

Performance for 4-MPD quantification was assessed on postmortem whole blood. Selectivity was evaluated on 5 independent blank blood samples: none of them showed interfering peaks at the retention times of 4-MPD or the internal standard. Calibration curves from 10.0 to 1,000 ng/mL were performed in triplicate. Linear regression with a 1/x weighting factor provided good linearity characterized by a determination coefficient (r^2) of 0.999. Precision and accuracy were determined on 5 replicates at 3 concentration levels: lower limit of quantification (LLOQ) at 10.0 ng/mL, low quality control (QC) at 100.0 ng/mL and high QC at 800.0 ng/mL. The dilution

process was assessed on 5 replicates of blood samples spiked at 1,200 ng/mL and diluted 10-fold in water. The precision and accuracy of the LLOQ, QCs and diluted samples (Table 1) met the acceptance criteria (14): accuracy within 15% and precision between 85% and 115%. Using the same procedure, 4-MPD was measured in the other fluid samples (bile, VH, urine) with matrix-matched calibration curves ($r^2 > 0.995$). Control samples in corresponding blank matrix at LLOQ, low and high QC and dilution levels displayed accuracy below 10% except for urine (LLOQ level: 13.4%, 10-fold dilution: 18.4%) and bile (LLOQ level: 16.3%). Samples exceeding the upper limit of quantification were reanalyzed after 2- to 10-fold dilution.

Table 1 Assessment of accuracy and precision of 4-MPD on postmortem whole blood (data from 5 replicates)

	Spiked concentration (ng/mL)	Found (mean +/- SD)	Precision (%)	Accuracy (%)
LLOQ	10	11.2 +/- 1.2	112.7	10.5
Low QC	100	98.5 +/- 6.5	98.5	6.6
High QC	800	721 +/- 42	90.1	5.8
Dilution (10-fold)	1200	1147 +/- 54	95.6	4.7

LLOQ: Lower limit of Quantification; QC: Quality Control; SD: Standard Deviation.

Metabolite screening

Femoral and cardiac blood, bile, VH and urine, extracted according to the 4-MPD procedure including acetylation, were injected on a GC (7890B GC system) coupled to a quadrupole combined to a time-of-flight detector (7200 Accurate Mass QTOF, Agilent Technologies). Based on general knowledge of synthetic cathinones' metabolic pathways (15–17), 4-MPD metabolites were hypothesized and screened for in the chromatograms by exact mass extraction. Identification was checked by comparing their mass spectra to the main fragmentation processes. Urine and bile samples were analyzed with and without enzymatic hydrolysis (beta-glucuronidase) in order to highlight any phase-2 metabolism.

Toxicological findings

Immunological screening in urine was positive for cocaine and negative for amphetamine, methamphetamine, cannabis, buprenorphine and opiates. Cocaine was quantified in femoral blood at 66 ng/mL along with its main metabolites: benzoylecgonine (BZE) and ecgonine methylester (EME) at 2,084 and 262 ng/mL, respectively. Neither ethanol nor alkyl nitrites were detected. In peripheral blood, GUS identified nevirapine (antiretroviral, not quantified), sildenafil (<1ng/mL) and bromazepam at 140 ng/mL, considered as therapeutic doses. GC-MS analyses of underivatized blood extract, using SWGDrug 2017 and Cayman 2017 libraries, identified 4-MPD, confirmed by injection of the commercial standard. Analysis of both right and left nasal swabs identified cocaine and 4-MPD. Concentrations of 4-MPD in autopsy samples are reported in Table 2.

Table 2 Concentrations of 4-MPD in postmortem fluids

Matrices	Concentration (ng/mL)
Peripheral blood	1285
Cardiac blood	1128
Left vitreous humor	734
Right vitreous humor	875
Bile	1187
Urine	> 10000 ^a

^a Outside calibration range (10-1,000 ng/mL) with 10-fold dilution

The 4-MPD metabolites suggested by GC-QTOF analysis and their metabolic pathways are represented in Figure 1. The mass spectrum and suggested fragmentation patterns of 4-MPD and metabolites (after acetylation) are available in Supplementary Data. 4-MPD (accurate molecular mass [M] 247.1572 g/mol, RT 9.19 min) seemed to undergo a reduction of the keto group and N-dealkylation, resulting in 3 metabolites detected in all postmortem samples: 1-dihydro-4-MPD (n° I, [M] 291.1834, RT 9.72), N-desmethyl-4-MPD (n° II, [M] 233.1416, RT 8.96), and 1-dihydro-N-desmethyl-4-MPD (n° III, [M] 277.1678, RT 9.36). 4'-hydroxymethyl-pentadrone (n° IV, [M] 305.1627, RT 11.29), obtained by hydroxylation of the benzylic carbon, was thought to have been detected in both urine and bile. A 5th metabolite, detected only in urine, was thought to be 4'-hydroxymethyl-N-desmethyl-pentadrone (n° V, [M] 291.1471, RT 11.07), resulting from 4'-methyl-hydroxylation and N-desmethylation, although this combined pathway was not previously reported for cathinones. As no standards are available, no quantitative interpretation could be made. By comparing bile and urine extracts with and without beta-glucuronidase hydrolysis, a significant increase in 4'-hydroxymethyl-pentadrone and, to a lesser extent, in 4'-hydroxymethyl-N-desmethyl-pentadrone was observed, seeming to indicate that these 2 metabolites could undergo phase-II metabolism, especially by conjugation to glucuronic acid.

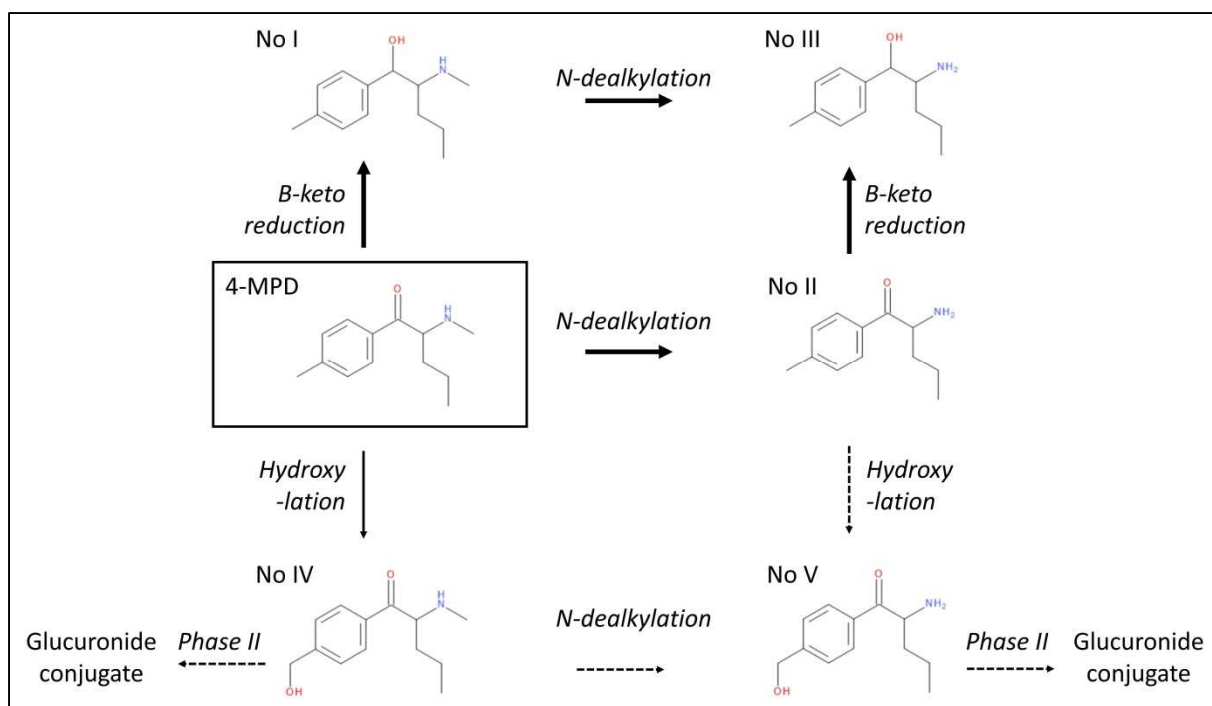


Figure 1: Presumed 4-methylpentadrone (4-MPD) metabolites and metabolic pathways.

No I: 1-dihydro-4-MPD, II: N-desmethyl-4-MPD, III: 1-dihydro-N-desmethyl-4-MPD, IV: 4'-hydroxymethyl-pentadrone, V: 4'-hydroxymethyl-N-desmethyl-pentadrone.

Discussion

4-MPD is the p-methyl analogue of pentadrone and a higher homologue of 4-methylmethcathinone (mephedrone). Except for being notified to warning systems and identified among illegal substances purchased from online vendors in Great Britain (18), few scientific data are available on this synthetic cathinone. Thus, user testimonies were searched for on the Internet. For NPSs, this approach enables a profile of the substance to be drawn up (19), although the unreliability of such data must be kept in mind. User testimonies are obviously influenced by inter-individual variability in drug sensitivity and tolerance and by personal expectations and past experience. In addition, users may be misguided as to the nature and/or purity of the substance, which can lead to contradictory reports. As an illustration, Hamby et al. reported an analysis of a free sample offered to clients by an Internet vendor, which was labeled as 4-MPD but was in fact chemically identified as N-ethyl-4-methylpentadrone (4-MEAP) (20). A close look at the Google trends history shows a peak of searches for “4-Methylpentadrone” and “4-MPD” between May and November 2016, mostly from Poland and Germany, and a slight interest coming from the USA and Brazil. Only 5 user discussions were found concerning 4-MPD, totaling 10 trip reports (21–25) between September 2013 and July 2020. Compared to the plethora of user testimonies available for some popular cathinones, the rarity of data concerning 4-MPD suggests relatively rare use.

The main administration route seems to be inhalation and vaporization (21–23). Inhalation is said to be associated with nose and throat burning (23) as severe as with ethylphenidate, 4-MPD sticking on the mucosae due to its poor solubility (24). Vaping can be difficult as the 4-MPD easily burns and the effect can be “harsh on the lungs” (24). Only one experience by intravenous injection was reported (25). One constant report is that oral

intake is inefficient (22–24): *"I tried three routes of admission (oral, inhalation, insufflation) several times and only inhalation seemed to stimulate a bit. Oral intake has been totally inefficient even with 100 mg."*

Concerning 4-MPD effects and interest, data from the scarce user reports were sometimes contradictory. It is described by some users as having a mild-to-strong stimulating effect (including sexual stimulation) (21–25), using a slightly higher dose than pentedrone (21), with a duration of stimulation around 2 hours (22,24). One user smoking 15 mg described immediate tachycardia (23). Some users, on the other hand, described it as barely active and of little interest (22,23). One described his experience as follows: *"It's quite stimulating. But not that euphoric nor empathic like some other cathinones."*

On the down side, users described sweating and reddening that can be considerable (24), and development of tolerance (22) and paranoid delusions (22,23) on repeated use: *"Definitely "moreish", subjects got mildly paranoid (but nowhere near the craziness that the "PV"-style [Pyrovalerone like, editor's note] drugs cause, [...] and somewhat horny; they had mild depression for a few hours after it was over. "*

The general impression coming from these reports is that poor efficacy as a stimulant and the adverse effects explain the relatively rare use of this substance: *"A conclusion you already get: it's worthless as a stim, you can easily find a better one."* (23)

To the best of our knowledge, no pharmacological evaluation or case reports involving 4-MPD use or toxicity were provided to help in interpreting its presence in the autopsy materials of the present case. Cathinone derivatives in general are powerful psychostimulants and acute toxicity mainly consists of neurological, cardiovascular and psychopathological symptoms such as paranoid psychosis, delirium symptoms, hyperthermia, rhabdomyolysis, renal failure, tachycardia, hypertension, cerebral edema and seizures (26,27). Numerous fatal intoxications implicated synthetic cathinones alone or in association with other drugs of abuse (28). By comparing blood levels of cathinones chemically related to 4-MPD encountered in such fatalities (28), the concentrations measured in the present case (1,285 ng/mL in femoral blood) can be considered high and implication in death can be suspected. Combined consumption with cocaine, another stimulant, could have a synergic pharmacological effect. The presence of bromazepam, a central nervous system depressant, even at therapeutic level, and of sildenafil, a potent vasodilator, may have partially contributed to the fatal event. Although a bottle of poppers was found in the victim's hand, no alkyl nitrites were detected, which, in no way, excluded consumption close to the death. Indeed, alkyl nitrites are highly volatile compounds that are known to undergo fast hydrolysis and metabolism (29). To the best of our knowledge, alkyl nitrites detection in biological matrices in a forensic context has never been reported in literature. These toxicological findings, associated with the macroscopic observations and absence of any alternative cause of death, led the pathologists to diagnose toxic death resulting from a combination of drugs of abuse (4-MPD and cocaine), plus administration of benzodiazepine.

The victim was found in a hotel room rented for the day, alongside numerous sex toys, with an open bottle of alkyl nitrites in his hand. Relatives' testimony reported previous experiences of drug consumption associated to sexual activity. In view of these elements and regarding the nature of the drugs, the consumption of cocaine, cathinone (4-MPD) and sildenafil could have been related to chemsex practice. Fatal intoxication has already been described in this context (9). Accidental overdose could result from experiencing new substances available on the Internet, implying lack of knowledge of the expected effects and toxicity and of appropriate use (dose, administration route, etc.). Users can also be misguided as to the nature or purity of substances, which can have

dramatic consequences. Repeated use of multiple substances during a party or a sexual encounter increases the risk of losing control of drug consumption, leading to overdose, especially as some drugs are associated with potent craving (i.e., strong desire to redose) (30).

Toxicologically, in the present case, 4-MPD was detected in all biological fluids. VH displayed the lowest concentrations, with VH/femoral blood ratios of 0.68 and 0.57 for right and left samples, respectively. Extensive excretion of the parent drug was observed in urine and, to a lesser extent, in bile. Investigation of 4-MPD metabolism in postmortem samples by high-resolution mass spectrometry identified 5 presumed metabolites. As showed by Uralets *et al.* (16), reduction of the keto group and N-dealkylation seemed to be the main metabolic pathways leading to 3 metabolites (n° I, II and III) present in all specimens. Two minor metabolites (n° IV and V) were also hypothesized. The present case exhibited a ratio between central blood and peripheral blood concentrations (C/P ratio) close to 1, seeming to indicate that 4-MPD is not prone to postmortem redistribution. 4-MPD and cocaine were detected in both right and left nasal swabs, which is compatible with consumption by sniffing as described for 4-MPD in user testimonies, although contamination of the nasal cavities by fluids cannot be ruled out (31).

Conclusion

This case reported the death of a 57-year-old man in circumstances suggesting chemsex practice. Autopsy findings indicated fatal intoxication mainly involving 4-MPD along with cocaine, sildenafil, bromazepam and nevirapine. 4-MPD is a synthetic cathinone poorly documented in the scientific literature. Only scarce user testimonies describe its use as a stimulant. Postmortem distribution in biological fluids was described for the first time, and metabolism was investigated. This case illustrates the toxicity of this pentedrone derivate and confirms the significant risk of overdose associated with chemsex practice.

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Supplementary data:

Mass spectrum and suggested fragmentation patterns of 4-MPD (after acetylation)

Mass spectrum and suggested fragmentation patterns of metabolite n° I (after acetylation)

Mass spectrum and suggested fragmentation patterns of metabolite n° II (after acetylation)

Mass spectrum and suggested fragmentation patterns of metabolite n° III (after acetylation)

Mass spectrum and suggested fragmentation patterns of metabolite n° IV (after acetylation)

Mass spectrum and suggested fragmentation patterns of metabolite n° V (after acetylation)