

## Postmortem Tissue Distribution of MDPV Following Lethal Intoxication by “Bath Salts”\*

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**3,4-Methylenedioxypyrovalerone (MDPV) is a psychoactive, synthetic analog of the central nervous system stimulant cathinone. Its recent popularity as a recreational drug in the United States has led to numerous reports to poison control centers across the country. As with other synthetic cathinones, the recreational use of MDPV has resulted in death. MDPV is thought to exert its pharmacologic effects by inhibiting the reuptake of dopamine and norepinephrine. This report describes the case of an exposure of a 39-year-old male to MDPV, which resulted in his death. Postmortem concentrations of MDPV in various tissues were measured. The detection of MDPV in tissues and fluids was accomplished using gas chromatography–mass spectrometry analysis after solid-phase extraction. Blood analysis also demonstrated therapeutic levels of lamotrigine, fluoxetine, risperidone, bupropion, pseudoephedrine and ibuprofen. The detection of cathinones in hair was conducted using high-performance liquid chromatography–tandem mass spectrometry after solid-phase extraction. MDPV was uniformly distributed among multiple tissues (blood, brain, muscle, cerebrospinal fluid and lung) at concentrations of approximately 0.4 to 0.6 µg/mL. Tissue and fluids responsible for detoxification/excretion had higher concentrations of MDPV (kidney, liver and bile > 0.8 µg/mL). A blood concentration ≥ 0.4 µg/mL was judged sufficient to cause death. The cause of death was ruled MDPV intoxication resulting in cardiac arrhythmia.**

### Introduction

Reports from emergency rooms indicate that use of synthetic cathinones (“bath salts”) have achieved epidemic status for abuse by young people in the United States (1–4). Multiple synthetic cathinones may be present in a single bath salt preparation. Until it was placed under Emergency Control as a Schedule 1 drug by the Drug Enforcement Agency (DEA) (5), 3,4-methylenedioxypyrovalerone (MDPV) was the most prevalent cathinone analogue present in bath salts. Other cathinones scheduled by the DEA were methylone and mephedrone. MDPV is a psychoactive, synthetic analog of the central nervous system (CNS) stimulants cathinone and pyrovalerone, and exerts its pharmacologic effect by inhibiting the reuptake of monoamine neurotransmitters, dopamine and norepinephrine (6). It has been classified as a mild entactogen and is snorted, smoked, taken orally or injected. Its relative potency is reported to be four times that of methylphenidate, with psychological effects lasting 3 to 4 h and physiological effects persisting for as long as 6 to 8 h. The most common signs of intoxication involved cardiovascular and neurological symptoms

including agitation, tachycardia, combative behavior, paranoia, insomnia, chest pain, myoclonus, hypertension, mydriasis, delusions/hallucinations, hyperthermia, seizures (3, 4), excited delirium syndrome and death (7). This is the second report of a death attributed to MDPV and describes the distribution of MDPV in tissues.

### Case History

A 39-year-old white male, with a history of schizophrenia, depression and drug abuse, was last known to be alive 4.5 h before being found by a family member face up in bed in an unresponsive state. Drugs that he had been prescribed at the time of his death included lamotrigine, fluoxetine, trazodone, bupropion, hydroxyzine, metformin, temazepam, risperidone and simvastatin. It was known by family members that he had begun snorting bath salts. All indications were that his exposure to bath salts occurred following the time he was last seen. A collection of bath salts and two unopened packages of synthetic cannabinoids (Demon and Flame) were found with the body. Empty jars of Tranquility and Infinity concentrated bath salts were present in a trash can near the body.

### Autopsy findings

Autopsy findings were unremarkable except for a mildly enlarged heart (430 g) and edematous lungs (right = 950 g, left = 710 g). Collected and analyzed specimens included femoral and heart blood, urine, cerebral spinal fluid, lung, kidney, liver, heart, psoas muscle, vertebral bone tissue, hair and six brain sections (frontal, parietal and occipital lobes, medulla, cerebellum, and lentiform nucleus). The decedent suffered from retinitis pigmentosa. Vitreous humor was not available for toxicology examination because his eyes were collected for study by the local eye bank. Bone and adipose tissue were found to be unsuitable for analysis.

### Materials and Methods

#### Chemicals and reagents

MDPV-HCl and methylone-HCl, both 1 mg/mL in methanol, were obtained from Lipomed (Cambridge, MA) and Cerilliant (Austin, TX). Mephedrone-HCl, 1 mg/mL in methanol, was obtained from Cerilliant. General screening for illicit and non-illicit drugs was performed using enzyme-linked immunoassay (ELISA) obtained from Immunalysis (Pomona, CA). Specific classes of drugs that were screened included opiates, oxycodone, methamphetamine, amphetamine, tricyclic antidepressants, carisoprodol and meprobamate, fentanyl, cannabinoids,

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cocaine and metabolites, barbiturates, benzodiazepines, phen-cyclidine and methadone.

Organic solvents [high-performance liquid chromatography (HPLC)-grade quality] were manufactured by Honeywell Burdick Jackson (Muskegon, MI) and purchased from Fisher Scientific. All other chemicals were of reagent grade quality or better and obtained from various commercial sources.

#### **Extraction and analysis of fluids and tissues**

MDPV and other basic drugs (including fluoxetine and lamotrigine, which were specific to this case) were extracted by solid-phase extraction (SPE) using UCT Clean Screen ZSDAU020 extraction columns (State College, PA), using a previously published basic drug extraction procedure provided by UCT (8). A whole blood matrix was used in the preparation of calibrators and controls for both blood and tissue samples; a urine matrix was used for the urine sample. Following appropriate dilution with distilled water, tissues were homogenized using a Waring blender. The study used an Agilent Technologies (Wilmington, DE) 6890/5973 gas chromatograph-mass selective detector (GC-MSD) with an Agilent 7683 auto-injector. The analytes were separated and detected using GC-MSD, identified by their characteristic retention times and quantitated using a calibration curve. Additional qualitative identification was made by ion ratio criteria. A Zebtron Guardian ZB-50 capillary column was used (#7CD-G004-08-GGT-C, 10 m  $\times$  0.18 mm i.d., 0.18  $\mu$ m film thickness) with a 2 m guard column and a helium gas flow rate of 1.2 mL/min.

Analysis of all basic drugs was performed in the full scan acquisition (EI) ionization mode. The ion source and quadrupole were set at 230 and 150°C, respectively, with a mass spectral range of  $m/z$  40–500 amu, detection threshold of 100 and scan rate of 14.02 scans/s. The quantitation of cathinones was performed using target ions for the following mass fragments:  $m/z$  126, 65, 149 ions for MDPV;  $m/z$  58, 135, 77 ions for mephedrone;  $m/z$  97, 58, 191 for the internal standard, methapyriline (target ions are in bold). All positive analyte spectra were compared to a spectral library or calibrator spectra for match quality. Retention times for analytes had a reference window of 2%.

The operating parameters were as follows: the injection port temperature was 250°C; the initial oven temperature was 100°C with an initial hold time of 2.75 min, a ramp rate of 20.0°C/min, a final temperature of 300°C with a final hold time of 4.25 min. The total run time was 17 min. The injector was operated in the pulsed/splitless mode at a temperature of 280°C with a ramped flow rate. The sample injection volume was 1  $\mu$ L. The limit of quantitation (LOQ) and upper limit of quantitation (ULOQ) for MDPV were 0.01 and 2.0 mg/L, respectively. The seven-point calibration curves were linear from 0.01 to 2.0 mg/L with coefficients of determination greater than 0.995. The coefficient of variation (CV) for intra-run precision was 12.7%. The CV inter-run precision was 5.88%.

Initially, specimens were sent to a reference laboratory (NMS Labs); the result of MDPV analysis from the reference lab was used as a control for validation of the MDPV calibration curve. The inter-laboratory results for the cardiac and femoral blood samples were within 4 and 17%, respectively.

#### **Extraction and analysis of hair**

Hair was washed with methanol before extraction. Hair was pulverized and 5 mg was transferred to a 13  $\times$  100 test tube. Acidified methanol (0.1%, v/v, HCl/MeOH) was added to the hair along with 50  $\mu$ L internal standards (MDPV- $d_8$ , mephedrone- $d_3$ , methylone- $d_3$ ; Cerilliant). The solution was heated at 60°C for 1 h and then centrifuged (3,500 rpm). The supernatant was decanted into a clean 13  $\times$  100 test tube and methanol was evaporated under nitrogen at 40°C. Two mL of 0.1 M sodium phosphate buffer (pH 6.0) was added to the tube and mixed by vortexing. Resuspended extracts were poured into ZSXE010 (XCEL 1) columns (UCT) and all effluents were eluted by gravity. The columns were washed with 1 mL of 100 mM sodium acetate buffer (pH 4.5) followed by 1.5 mL methanol and then allowed to dry for five minutes at a positive pressure >10 in. Hg. An elution solvent (2 mL) of dichloromethane-isopropanol-ammonium hydroxide (78:20:2) was used to elute drugs from the column. The eluate was evaporated to dryness under nitrogen at 40°C and reconstituted with 100  $\mu$ L of mobile phase A (0.1% formic acid in HPLC-grade water).

A six-point calibration curve from 0 to 500 pg/mg was used for quantitation. The methanolic extract was diluted 1:10 so that the concentration of MDPV fell within the range of the calibration curve.

#### **Apparatus**

The LC-tandem mass spectrometry (MS-MS) system consisted of an Agilent 6430 LC triple quadrupole mass spectrometer (Santa Clara, CA) operated in electrospray ionization (ESI) in positive mode, and an Agilent 1290 Infinity HPLC system.

#### **HPLC conditions**

Chromatography was performed using an Agilent Zorbax Eclipse Plus C18 LC column (2.1  $\times$  50 mm  $\times$  1.8  $\mu$ m) with gradient elution. The mobile phases consisted of 0.1% formic acid in HPLC-grade water (eluent A) and HPLC grade acetonitrile (eluent B). The flow rate was 0.4 mL/min. The mobile phase gradient was programmed as follows: 0.01–3.00 min, eluent B increased from 0 to 75%; 3.00–3.01, eluent B increased from 75 to 100%; 3.50–3.51, eluent B decreased from 100 to 0% to re-equilibrate the column.

The injection volume was 10  $\mu$ L and the column temperature was maintained at 50°C. The autosampler was operated at 8°C and the autosampler needle was rinsed with 85% methanol–15% deionized water for 10 s.

#### **MS-MS conditions**

The mass spectrometer in ESI mode was operated with nitrogen gas under the following conditions: temperature, 325°C; gas flow rate, 8 L/min; nebulizer gas pressure, 40 psi; capillary interface voltage, 4,000 V; delta EMV + 600; the polarity was positive. All analyte-specific settings were auto-optimized.

## Validation of hair analysis

### Limit of detection and quantitation

The LOD was established as the lowest concentration that produced a signal-to-noise (S/N) ratio > 3 and was determined to be 2.0 pg/mg for all analytes. The LOQ was defined as the lowest concentration that was quantitated with a precision of less than 20% CV ( $n = 9$ ). The LOQ of hair was determined to be 2.0 pg/mg.

### Linearity

The calibration curves proved linear over the range of 2–3,000 pg/mg of hair. A coefficient of determination of 0.999 or higher was obtained for all curves.

### Precision and accuracy

The precision for all compounds was below 15% CV for all levels, except the LOD, which was below 20. The deviation was less than 10% for all levels above the LOD, except 500 pg/mg (deviation < 13%).

### Extraction recoveries, matrix effects and process efficiencies

The extraction recoveries (ERs), matrix effects (MEs) and process efficiencies (PEs) were estimated with a set of three different samples at a concentration of 50 pg/mg. The ME was  $\leq 20\%$  for all compounds except methcathinone (+81%). The ERs and PEs were all greater than 95%.

## Results

The concentrations of MDPV among tissues are shown in Table I.

MDPV was fairly uniformly distributed among multiple tissues at a value of approximately 0.4 to 0.5  $\mu\text{g/mL}$ . The ratio of heart to peripheral blood gave no clear indication that MDPV undergoes postmortem redistribution. Levels in different brain locations reflected a higher concentration in parietal (20%), cerebellum, medulla and occipital regions (40%) than in the frontal and lentiform nucleus. Whether this is a true reflection of MDPV distribution with pharmacological significance or simply a result of inter-specimen analytical variability is not known.

Tissue and fluids responsible for detoxification/excretion had higher concentrations of MDPV (kidney, liver and bile > 0.8  $\mu\text{g/mL}$ ). Other drugs identified in blood [also by a reference laboratory (NMS Labs)] included caffeine, fluoxetine (0.29  $\mu\text{g/mL}$  in blood), lamotrigine (< 0.4  $\mu\text{g/mL}$ ), risperidone and hydroxyrisperidone (6.8 and 6.3 ng/mL, respectively), ibuprofen, nicotine/cotinine, pseudoephedrine (130 ng/mL) and benzotropine (10 ng/mL). Nicotine, cotinine, pseudoephedrine, m-chlorophenylpiperazine and methylone were present in urine. All substances detected in blood, which were known to have been prescribed, were at concentrations consistent with or below the prescribed doses.

MDPV was present in all tissues analyzed, including hair. Both MDPV and methylone were detected in hair at 11,660 and 1,332 pg/mg, respectively. Mephedrone and methcathinone were not detected in the hair sample. The presence of MDPV in hair indicates that this was not the decedent's first exposure

**Table I**

Toxicology Results for MDPV Overdose

| Source              | Drugs detected ( $\mu\text{g/mL}$ or $\mu\text{g/g}$ ) |             |
|---------------------|--------------------------------------------------------|-------------|
|                     | MDPV                                                   | Methylone   |
| Femoral blood       | 0.44                                                   |             |
| Heart blood         | 0.50                                                   |             |
| Urine               | > 5.0                                                  | Positive    |
| Gastric             | > 2.0 = 50 mL                                          |             |
| Bile                | 0.88                                                   |             |
| Cerebrospinal fluid | 0.41                                                   |             |
| Lung                | 0.60                                                   |             |
| Kidney              | 0.84                                                   |             |
| Liver               | 0.98                                                   |             |
| Muscle              | 0.56                                                   |             |
| Spleen              | 0.64                                                   |             |
| Brain               |                                                        |             |
| Parietal            | 0.36                                                   |             |
| Cerebellum          | 0.42                                                   |             |
| Lentiform nucleus   | 0.30                                                   |             |
| Frontal             | 0.30                                                   |             |
| Occipital           | 0.42                                                   |             |
| Medulla             | 0.42                                                   |             |
| Heart               | 0.12                                                   |             |
| Hair                | 11,660 pg/mg                                           | 1,332 pg/mg |

and is consistent with the report from family members that he had begun snorting bath salts. Hair was not segmented to determine when the use of bath salts might have begun. Based on the structural similarity of MDPV to sympathomimetic amines and because symptoms of MDPV exposure include vasoconstriction, hypertension and tachycardia, a plausible mechanism of death in this case was cardiac arrhythmia.

## Discussion/Conclusion

This is the second report of a death attributed solely to MDPV exposure. In a recent report (7), the blood level determined upon hospital admittance was 0.08  $\mu\text{g/mL}$ ; the individual's condition progressively declined to brain death over the next two days. Levels of MDPV in other tissues were not reported. The current case appears to be an acute death from MDPV, resulting from recent experimentation with bath salts. The determination of the cause of death was a diagnosis of exclusion in that the decedent was an otherwise healthy male with no other apparent reason to die. MDPV was distributed among multiple tissues with values ranging from 0.12  $\mu\text{g/mL}$  (heart) to 0.98  $\mu\text{g/mL}$  (liver). Other drugs present at therapeutic levels were lamotrigine, fluoxetine, risperidone, ibuprofen, benzotropine and pseudoephedrine. These drugs had been prescribed and taken by the decedent for an extended period, so pharmacologic acclimation to these substances would have occurred. The effect of low levels of multiple drugs on MDPV toxicity is not known. MDPV is reportedly metabolized by CYP2C19 and possibly CYP1A2 and CYP2D6 (9), risperidone by CYP2D6 and CYP3A4 (10, 11), whereas lamotrigine is metabolized primarily by glucuronic acid conjugation to inactive metabolites (12). The metabolism of pseudoephedrine to norpseudoephedrine is very limited, with 88% of a dose excreted unchanged in the urine (13). Fluoxetine is a substrate for and a potent inhibitor of CYP2D6 and a moderate inhibitor of CYP1A2, CYP2B6 and CYP2C9/2C19 (14). Whether fluoxetine increased the toxicity of MDPV by preventing its metabolism is not known.



At the present time, there has been one other report of death from MDPV in the scientific literature (7). This individual experienced a delayed death (42 h after initial presentation) with final sequelae of anoxic brain injury, rhabdomyolysis, coagulopathy and acidosis. The onset of intoxication was characterized by agitation and aggression, removal of clothing, incomprehensible yelling, considerable strength, hyperthermia and cardiac arrest. The stage of intoxication was characterized as MDPV-induced excited delirium. The analysis of the earliest blood and urine samples obtained from a tertiary care center demonstrated 0.82 and 0.67 µg/mL in blood and urine, respectively.

Fatal intoxications and excited delirium have been described with other bath salts. Mephedrone (4-methylmethcathinone) exposure produced violent behavior in a 36-year-old male, resulting in lacerations, bruises and minor brain swelling (non-life-threatening) (15). Following autopsy, his femoral blood concentration for mephedrone was 5.1 µg/mL. Other deaths directly attributed to mephedrone demonstrated femoral blood concentrations of 0.98 and 2.24 µg/mL (16). Levels of methylone in four postmortem cases have recently been described (17). The only death directly attributed to methylone intoxication found the following levels in fluids and tissues (mg/L or mg/Kg): central blood, 0.74; peripheral blood, 0.67; urine, 38; liver, 1.8; kidney, 2.3; spleen, 2.1; bile, 1.8. Another case in the same paper, in which the decedent died of blunt force trauma and drowning after a high-speed chase, characterized both methylone and MDPV. MDPV and methylone concentrations (mg/L or mg/Kg), respectively, were: heart blood, 0.47 and 0.06; liver, 0.53 and 0.14; kidney, 0.49 and 0.16; bile, 0.58 and 0.42.

Based on the present case, the lethal level of MDPV is judged to be  $\geq 0.4$  µg/mL in peripheral blood. Additional case reports are needed to better characterize the lethal level of MDPV intoxication.

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