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False-positive phencyclidine immunoassay results caused by 3,4-methylenedioxypyrovalerone (MDPV)

Dear Editor,

In the July 2011 issue of *Drug Testing and Analysis*, Bell *et al.* reported that the synthetic cathinone derivative 2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one ('butylone') produced a positive screening result on the Microgenics CEDIA Amphetamines/Ecstasy immunoassay.[1] We report false-positive phencyclidine (PCP) immunoassay results caused by 3,4-methylenedioxypyrovalerone (MDPV) (Figure 1), another psychoactive synthetic cathinone derivative that is sold and deceptively marketed as Bath Salts.[2–6]

In April 2011, a male abusing psychoactive Bath Salts presented to a western Maryland emergency department with psychotomimetic and sympathomimetic toxicity. Within hours, his clinical course rapidly deteriorated as severe hyperthermia ensued and he did not survive. Of note, the hospital's chemistry laboratory reported that screening immunoassay testing of ante mortem urine was reactive for PCP (Synchron immunoassay system on a Beckman Coulter DxC 800 Instrument); however, confirmatory analytical testing for PCP was not performed, and the residual urine specimen was not submitted to the medical examiner's office. The medical examiner reported that gas chromatography–mass spectrometry (GC-MS) analysis of post-mortem blood did *not* detect PCP; instead, MDPV was detected at a concentration of 1.0 mg/L in peripheral blood.

One of the authors (AMM) reported the case to both Beckman Coulter and ThermoFisher and underscored that a number of hospital-based toxicology laboratories were *not* performing confirmatory PCP testing of specimens that screened immunoassay reactive for PCP. To determine if the presence of MDPV can produce a false-positive PCP immunoassay result by the Synchron system, the author requested that an MDPV 'spiking' experiment be conducted at ThermoFisher's California laboratory using the PCP reagent manufactured for Beckman Coulter by ThermoFisher on the Beckman Coulter DxC analyzer. The immunoassay system is calibrated to detect PCP at concentrations greater than 25 ng/ml. A 1 mg/ml MDPV standard was purchased by ThermoFisher from Cerilliant (Part # M-146; Lot # FN 021811–01). A 0.5 mg/ml sub-stock standard was prepared by quantitatively adding 0.5 ml of the 1 mg/ml stock to 0.5 ml of negative control urine. Four test samples were then prepared by serial dilution of the 0.5 mg/ml sub-stock, resulting in concentrations of 0.25 mg/ml, 0.125 mg/ml, 0.063 mg/ml, and 0.031 mg/ml. The samples were analyzed on the DxC analyzer according to the manufacturer's recommended protocol.[7] All four samples produced positive results. Testing continued to determine the lowest MDPV concentration that would still produce a positive result. Two additional dilutions at 1:5 and 1:10 of the 0.031 mg/ml were prepared, resulting in final concentrations of 0.0062 mg/ml and 0.0031 mg/ml. The 0.0031 mg/ml sample produced a rate reading of 451.7 just below the 25 ng/ml cut-off level with the rate reading of 465.7; the 0.0062 mg/ml sample produced a positive result with the rate reading of 498.5 (Table 1). It was evident that a concentration of MDPV greater than 0.0031 mg/ml (3100 ng/ml) would produce a positive response on the Beckman Coulter Synchron PCP immunoassay system. A mirror-image spiking experiment using another synthetic cathinone derivative 4-methylmethcathinone ('mephedrone') did *not* elicit a positive PCP immunoassay result.

Further investigation revealed that abusers of psychoactive Bath Salts in multiple states were presenting with reactive PCP screening immunoassay results. It was observed that in eastern North Carolina, abuse of PCP is a rare event, similar to the situation in western Maryland. During January, February, and March 2011, 12 Bath Salts abusers experiencing psychotomimetic and sympathomimetic toxicity were hospitalized on the psychiatry service of a community hospital in eastern North Carolina. All 12 patients underwent urine drug testing (Abbott FPIA system), and 5 of the specimens screened positive for PCP. An expanded investigation discovered that from April 2010 through June 2011, a total of 48 symptomatic Bath Salts abusers treated in the eastern North Carolina community hospital had screened immunoassay positive for PCP. None of these 48 urine specimens underwent targeted confirmatory analytical testing for PCP. However, samples of psychoactive Bath Salts products identified to be abused by a number of patients (labelled as 'White Horse' and 'Cloud Nine') were purchased by hospital staff and referred for analysis to NMS Labs in Pennsylvania (Forensic Science Department, Willow Grove, PA); high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis identified the substance in these Bath Salts products to be MDPV.[8]

A number of states' Department of Corrections Probation and Parole Divisions informed one of the authors (AMM) that they had also noted a recent unexplained surge in PCP-positive screening immunoassay test results. In September 2011, this author requested that five urine specimens that had been collected from persons on probation or parole and screened positive-for-PCP on the DRI PCP immunoassay undergo further analysis. In ThermoFisher's laboratory, targeted GC-MS analytical testing of these five urine specimens did *not* detect PCP. Further analysis by LC-MS/MS at NMS Labs detected MDPV in three of the

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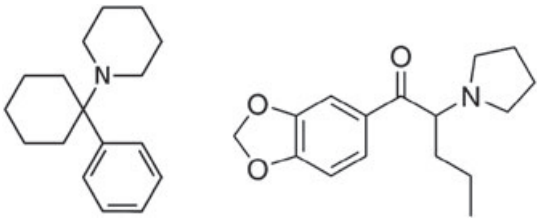

PCP MDPV

Figure 1. Chemical structures of PCP and MDPV.

five specimens at concentrations of 720 ng/ml, 7400 ng/ml, and 32000 ng/ml (reporting cutoff limit for MDPV 100 ng/mL). Because word-on-the-street and Internet websites assure substance abusers that use of synthetic cathinone derivatives is *not* detected by conventional drug screening tests (i.e. immunoassays), psychoactive Bath Salts products are desirable for individuals who must undergo routine urinalysis toxicology screenings, such as those in drug courts or on probation and parole. Those on probation and parole selectively abuse psychoactive Bath Salts believing that they can avoid detection of their surreptitious ongoing substance abuse.[9]

As a pyrrolidinophenone, MDPV is more lipophilic than non-pyrrolidine cathinone derivatives. The high lipophilicity of MDPV is caused by the pyrrolidine ring and the tertiary amino group creating a less polar molecule more able to cross the blood–brain barrier.[10–12] As a potent monoamine uptake inhibitor, MDPV causes intense cravings to binge and re-dose, and the development of tolerance and dependence causes abusers to consume extraordinary amounts of MDPV in attempts to obtain desired psychoactive effects. At the September 2011 Joint Meeting of the Society of Forensic Toxicologists and International Association of Forensic Toxicologists, Alexy *et al*. at NMS Labs reported that among 291 clinical urine specimens submitted for MDPV testing (LC-MS/MS; reporting limit 100 ng/ml) from January 2011 to mid-July 2011, 59 of the specimens were positive for MDPV, and in 57 a quantitative result was determined. Results ranged from 100 ng/ml to 67000 ng/ml; the mean (average) was 4345 ng/ml.[13] In October 2011, the US Department of Justice Drug Enforcement Administration reported that individuals have consumed up to *five grams* of synthetic cathinones per session (i.e. repeated administration and binging) in order to prolong the duration of psychoactive effects, to satisfy a craving, or to satisfy a strong urge to re-dose.[14] It is clear that substance abusers are consuming MDPV in amounts high enough to produce false-positive PCP immunoassay results (i.e. MDPV urine levels > 3100 ng/ml).

In the March 2012 issue of the *American Journal of Drug and Alcohol Abuse*, Kasick *et al*. in Ohio report the clinical case of a male who was abusing psychoactive Bath Salts ('Arctic Blast') and developed psychotomimetic and sympathomimetic toxicity.[15] The hospital's toxicology laboratory reported that screening immunoassay testing of the patient's urine was reactive for PCP (UniCel DxC 800 System Homogeneous Enzyme Immunoassay Method, Beckman Coulter); however, targeted confirmatory testing of the urine specimen did *not* detect PCP. The hospital's toxicology director reported 'several other similar recent unexpected cases of initial presumptive positive PCP screens that were not reproducible through further confirmatory testing'. Kasick *et al*. suggested that the synthetic cathinone derivative abused by their patient was 'mephedrone'; however, they did not perform analytical toxicology testing to identify which specific synthetic cathinone derivative was present in their patients' biological fluids, or in the psychoactive Bath Salts product consumed by their patient. We believe that their hospital's recent unexpected surge of false-positive PCP immunoassay results was caused by patients' abuse of MDPV-Bath Salts.

A number of heterogeneous compounds have been reported to interfere with screening immunoassay PCP testing (e.g. dextromethorphan; diphenhydramine; doxylamine; ibuprofen; imipramine; ketamine; meperidine; mesoridazine; thioridazine; tramadol; venlafaxine).[16–18] We suspect that a number of immunoassay systems may also be prone to false-positive PCP results caused by MDPV. Furthermore, a number of MDPV metabolites have been identified,[19,20] and it remains to be determined whether any of these metabolites contribute to MDPV's cross-reactivity with PCP immunoassays. If yes, then the MDPV concentration required to produce a reactive PCP immunoassay result may be less than the above MDPV spiking experiment indicates (> 3100 ng/ml); note the aforementioned 'probation and parole' clinical urine specimen that screened immunoassay-false-positive-for-PCP and contained MDPV at a concentration of 720 ng/ml.

As abuse of MDPV continues and additional designer compounds enter the psychoactive drug abuse arena, we recommend that *all* positive PCP screening immunoassay results be confirmed using additional analytical methodologies (e.g. GC-MS; LC-MS/MS).

Yours,

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Table 1. Results (expressed in rate units) of MDPV spiking experiment on synchron PCP immunoassay*

25 ng/mL Cutoff	mg/mL MDPV						
	0.500	0.250	0.125	0.063	0.031	0.0062	0.0031
464.00	556.87	593.17	593.74	589.57	575.88		
465.7						498.5	451.7

* Testing was performed on two separate runs.

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