Pathological Findings in 2 Cases of Fatal 25I-NBOMe Toxicity

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Abstract: The research compound 25I-NBOMe, also known as CIMBI-5 or INBMeO, was created in academic laboratories as a potent serotonin 2A receptor agonist. Because of its high affinity and ambiguous legal status, recreational drug enthusiasts have used this compound as a powerful alternative to other hallucinogenic drugs such as lysergic acid diethylamide. We report 2 deaths after 25I-NBOMe ingestion by decedents who attended separate "rave" parties. The first case involved a 21-year-old male who admitted taking "acid" to his friend. A sudden violent rage caused him to flail about, and he subsequently became unresponsive. The postmortem examination revealed numerous external injuries that were consistent with physical aggression. The second case involved a 15-year-old female who was socializing outside a rave party, became ill, and rapidly deteriorated as her friend transported her to the hospital. The postmortem assessment was similar to the first case in that external contusions featured prominently. Comprehensive toxicology screens in both cases revealed only evidence of marijuana use. A deeper analysis using time-of-flight mass spectrometry revealed the presence of 25I-NBOMe, which was further confirmed by tandem-mass spectrometry. The behavior and injuries in these cases reveal a consistent pattern preceding fatal 25I-NBOMe toxicity.

Key Words: 25I-NBOMe, CIMBI-5, 2C-I NBOMe, synthetic designer drug

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The incidence of research compounds misused as recreational drugs has risen dramatically in recent years. Novel synthetic compounds such as cannabinoids, cathinones, and phenethylamines serve as neurochemical modifiers commonly known as "Spice," "Bath Salts," and "Legal LSD," with the latter conferring a new experience in psychedelic hallucinations.[1] 25I-NBOMe, chemically known as 2-(4-iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine and also known as CIMBI-5, INBMeO, or "N-Bomb," is a molecular tool for neurobiology experiments modeled upon 2C-I[2] that was synthesized in the search for more potent and selective serotonin receptor agonists.[3–6] Because of its high affinity for the serotonin 2A receptor, it has gained popularity among drug enthusiasts as a powerful yet structurally dissimilar substance, and therefore it was perceived as a legal alternative to other hallucinogenic drugs such as lysergic acid diethylamide (LSD) (Fig. 1). Because 25I-NBOMe has not been tested or approved for use in humans, its effects or outcomes are unknown.

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We report 2 deaths due to 25I-NBOMe, where the decedents were attending "rave" parties before the terminal events. The first case involved a 21-year-old male driver who had admitted taking "acid" to his passenger. A sudden surge of violent behavior caused him to pull over and destroy the interior of the car, and he then became unresponsive. The postmortem examination was unremarkable internally despite numerous external superficial injuries consistent with physical aggression. The second case involved a 15-year-old female who was socializing outside a rave party, became ill, and rapidly deteriorated as friends transported her to the hospital. The postmortem assessment was similar to the first case in that external contusions featured prominently, whereas internal examinations revealed that the injuries were merely superficial. Comprehensive toxicological screens in both cases exhibited only evidence of marijuana use. A deeper analysis using time-of-flight mass spectrometry revealed the presence of 25I-NBOMe, which was further confirmed by tandem-mass spectrometry.

METHODS

Screening for 25I-NBOMe was performed by liquid chromatography time-of-flight mass spectrometry. The details of the instrument settings and extraction procedure have been described elsewhere.[7] Briefly, a liquid-liquid extraction was used to remove and concentrate drug residue from the postmortem heart blood. The molecular formula of 25I-NBOMe is $C_{18}H_{22}INO_3$, which yields an exact mass of 427.0644 g/mol. Under acidic conditions for positive mode electrospray ionization, the reference standard of 25I-NBOMe (Cayman Chemical, Ann Arbor, Mich) has an observed 428.0719 m/z mass-to-charge ratio (428.0717 m/z theoretical) at a retention time of 5.63 minutes.

The confirmation of 25I-NBOMe was accomplished by liquid chromatography tandem mass spectrometry, a comparably more sensitive and specific technique. In this assay, 0.5 mL of blood and urine specimens were analyzed by combining with 10 ng of 3,4-methylenedioxymethamphetamine-D5 (Cerilliant, Round Rock, Tex) as a deuterated internal standard. A liquid-liquid extraction was performed, consisting of 2 mL ethyl acetate (Sigma Aldrich, St Louis, Mo) and 0.2 mL of a saturated alkaline (pH 12) sodium borate buffer. After vortexing and centrifugation, the organic layer was removed to a fresh test tube and acidified with 10 μL of concentrated hydrochloric acid (Sigma Aldrich). The solvent was evaporated with compressed nitrogen, and the residue was resuspended in 1 mL of a 7 mM ammonium formate/10% acetonitrile buffer.

Liquid chromatography used a 3.5-μm Zorbax C18 column (Agilent Technologies, Santa Clara, Calif) measuring 3.0 × 100 mm with 7 mM ammonium formate mobile phase A and 100% acetonitrile mobile phase B. The flow rate was set at 0.25 mL/min at a temperature of 50°C. The gradient profile was begun at 10% B from 0 minutes, reaching 20% by 2 minutes. The gradient was increased to 95% by 5 minutes and held until 6.5 minutes. The

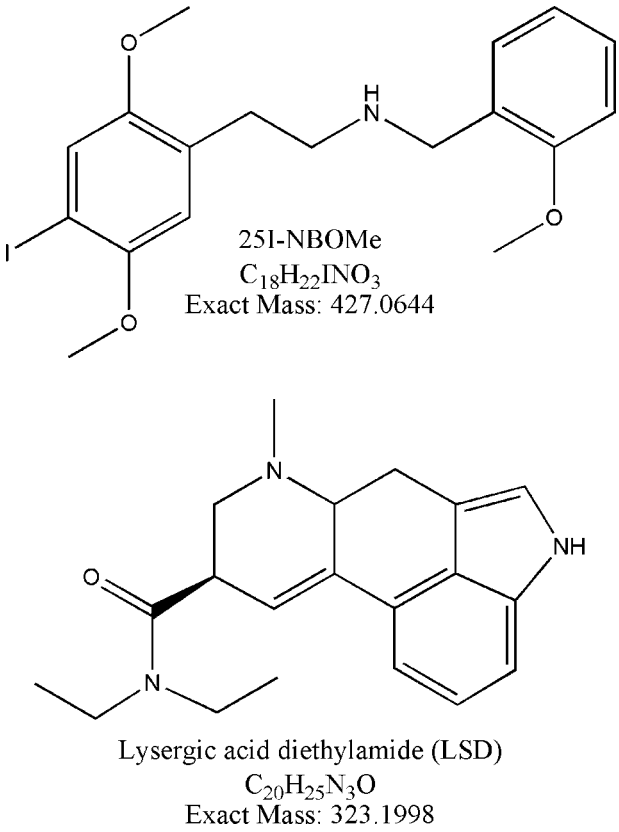

FIGURE 1. Structure of 25I-NBOMe compared with LSD. The compounds show little similarity but share common targets.

gradient was returned to 10% B by 7 minutes and then continued another 5 minutes for washout and regeneration of the column.

Tandem mass spectrometry was operated in positive ion mode with gas temperature at 320°C and a flow rate of 6 L/min. As mentioned earlier, the protonated form of 25I-NBOMe has a nominal mass of 428 m/z. A reference standard of 25I-NBOMe was infused as a positive control, and the following transitions were monitored: 428 to 121 and 428 to 91, using 16 V and 48 V collision energies, respectively. In order to track the internal standard 3,4-methylenedioxymethamphetamine-D5, acquisition was set for transitions of 199 to 165 and 199 to 136, using collision energies of 15 V and 20 V, respectively. Under these conditions, 25I-NBOMe eluted at 8.26 minutes, and the internal standard eluted at 6.22 minutes.

RESULTS

Case 1

This 21-year-old white male had no significant medical history but was known to engage in daily marijuana use. On the night of his death, he attended a rave party with a friend and admitted that he had taken 2 "hits of acid." In addition, both men smoked marijuana. After leaving the rave, the decedent and his friend ate a meal and were traveling home when the decedent, who was driving the vehicle, began to hallucinate and flail about, striking the friend and ripping the stretched piercing jewelry out of his own earlobes. The friend helped pull the vehicle to the side of the road; while the decedent continued to punch the console and rip accessories off of the interior of the vehicle, the friend exited the vehicle in search of a road sign location while calling emergency services. When he returned, he found the unresponsive decedent upside down in front of the vehicle with his head against the passenger side floorboard, his back arched over the console, and his right leg resting on the driver side seat headrest. The friend removed the decedent from the vehicle and initiated cardiopulmonary resuscitation. However, the decedent was pronounced dead upon the arrival of emergency services.

Scene examination revealed damage to the steering wheel and driver seat. The windshield wiper and the turn signal indicator levers were broken off (Fig. 2), as was the seat depth lever. The rubber gasket encircling the sun roof had been ripped from its seating and was discovered outside of the vehicle along with the keys. The contents of the center console were overturned, and items of clothing were found scattered throughout the inside of the vehicle. Vomit was present on the floorboards and both front seats.

At autopsy, external examination findings included numerous scattered, linear, and confluent contusions and ecchymoses of the forehead, frontal scalp, and face (Fig. 3); anterior and posterior shoulders and chest (Fig. 4); bilateral upper and lower extremities, most prominently on the dorsal upper extremities, anterior lower extremities, and knees (Fig. 5); hips; and heels. Gaping piercings of both earlobes with surrounding erythema were present. Few faint petechial hemorrhages were seen on the palpebral surfaces of the conjunctivae.

The internal examination revealed numerous areas of hemorrhage within the subcutaneous tissue, which corresponded to

FIGURE 2. Case 1, Vehicle control damage caused by the intoxicated driver after 25I-NBOMe ingestion.

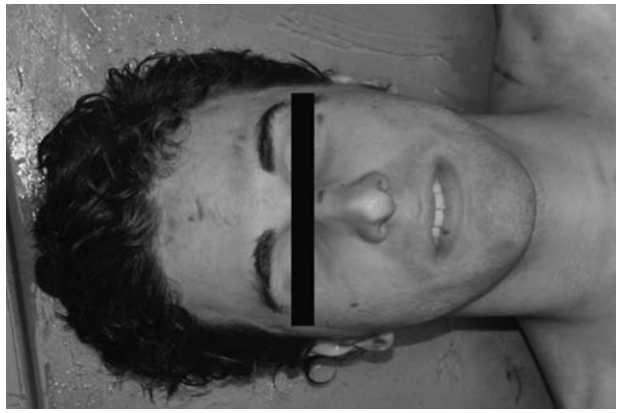

FIGURE 3. Case 1, External examination showing abrasions on the forehead and face.

the areas of cutaneous contusions. Soft tissue hematomas were identified in the subscalpular right temporal region, in the right temporalis muscle (Fig. 6), over the fascia of the left temporalis muscle, in the subcutaneous tissue over the lumbar fascia of the lower back, and in the left anteromedial lower leg (Fig. 7). Scattered petechiae were discovered on the epicardial surface (Fig. 8). Aspirated gastric contents were identified in the upper and lower airways. The lung parenchyma was moderately congested and edematous (right lung, 675 g; left lung, 660 g). No bony fractures, contusions or lacerations of the thoracic or abdominal organs, or hemorrhages within the body cavities were identified. Aliquots of heart blood and urine were submitted for analysis, confirming the presence of 25I-NBOMe in addition to a marijuana metabolite.

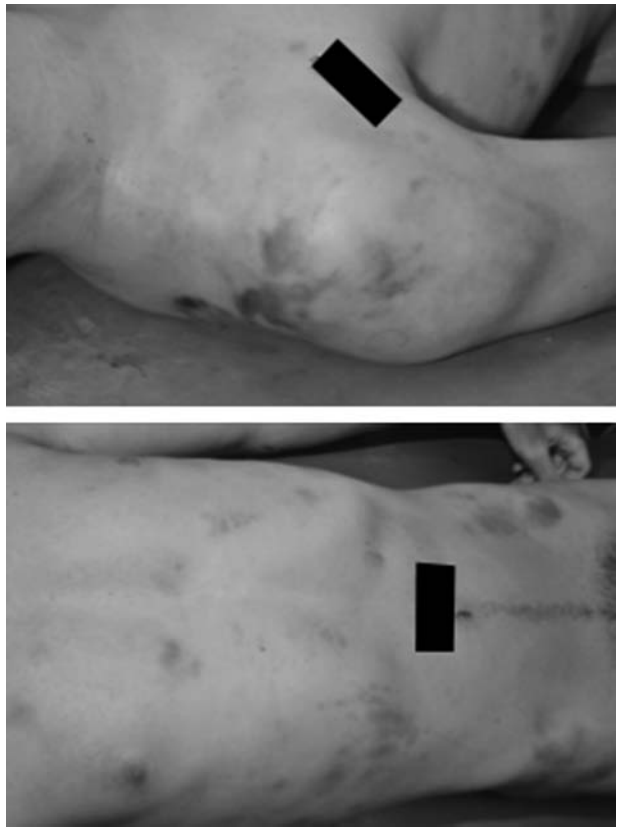

FIGURE 4. Case 1, Ecchymoses across shoulders and torso.

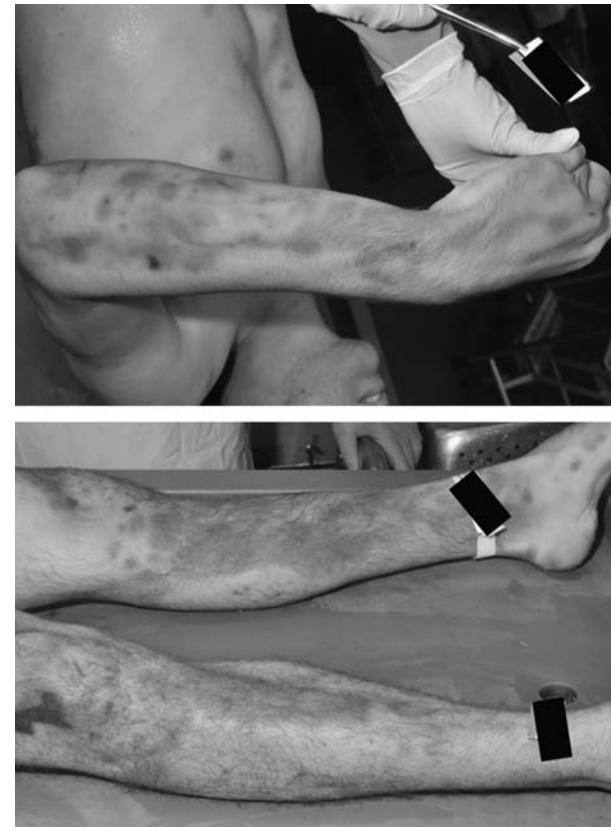

FIGURE 5. Case 1, Numerous scattered, linear, and confluent contusions of the upper and lower extremities.

Case 2

This 15-year-old white female had a history of marijuana and 3,4-methylenedioxymethamphetamine use but no other significant medical history. On the night of her death, she went with a friend to a rave party, but she was denied admittance

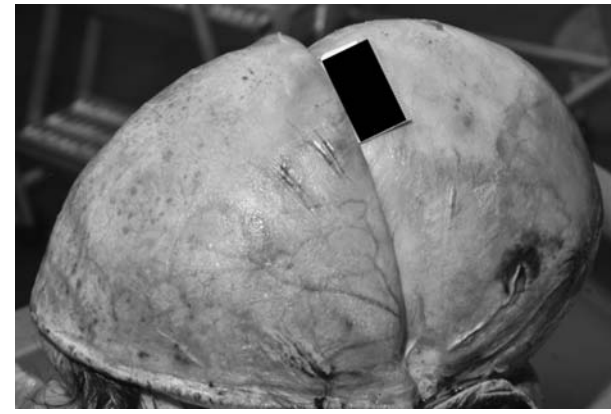

FIGURE 6. Case 1, Hematoma in the subscalpular right temporal region.

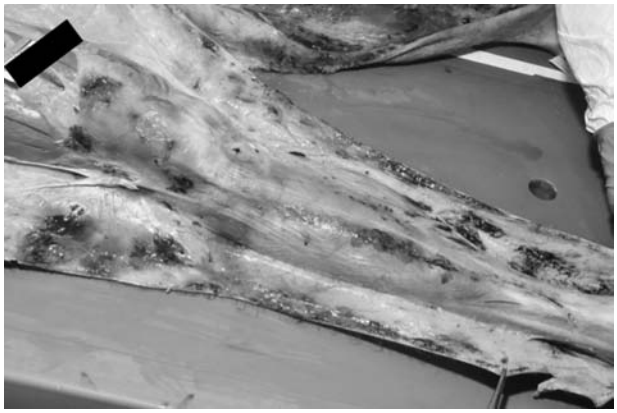

FIGURE 7. Case 1, Distinct soft tissue hematomas in the anteromedial lower leg.

because of her young age. The decedent remained outside in the parking lot where others were socializing, while the friend briefly entered the party. When the friend returned, the decedent had apparently ingested an unknown clear liquid. While being driven home from the party by her friend, the decedent complained that she did not feel well, began flailing her arms and legs, and subsequently became unresponsive. The friend drove the decedent to a local hospital. On arrival, the decedent was in asystole with a rectal temperature of 39.9°C. She was intubated, and advanced cardiac life support protocol was initiated. The decedent was pronounced dead approximately 18 minutes after arrival.

At autopsy, external examination findings included isolated palpebral petechial hemorrhages of the conjunctivae (Fig. 9). An endotracheal tube was in place, and white foam filled the endotracheal tube (Fig. 10) and the oropharynx. Numerous areas of abrasion and contusion were identified over the shoulders and upper extremities, left hip, right buttock, left thigh, and shins (Fig. 11).

Internal examination revealed areas of subscalpular hemorrhages in the left frontoparietal, right parietal, and right occipital regions (Fig. 12). The trachea and bronchi contained copious amounts of white foam. The lung parenchyma was moderately congested (right lung, 575 g; left lung, 500 g). No bony fractures, contusions or lacerations of the thoracic or abdominal organs, or hemorrhages within the body cavities were

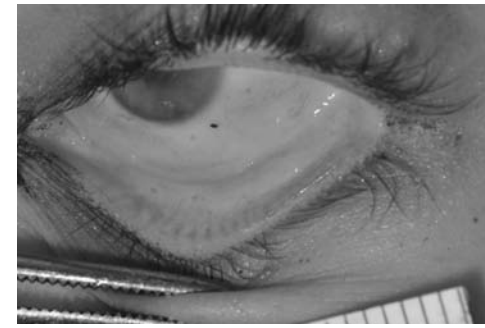

FIGURE 9. Case 2, Faint petechial hemorrhages of the conjunctivae.

identified. Aliquots of heart blood and urine were submitted for analysis, which confirmed the presence of 25I-NBOMe and trace amounts of marijuana metabolite. No other substances were detected.

DISCUSSION

This is the first forensic pathological characterization of fatalities proven to involve 25I-NBOMe. In the external examination, both of the decedents presented with widespread contusions on the head, torso, and extremities, as well as conjunctival petechial hemorrhages.[8] Without well-characterized scenes and events, these injuries could be consistent with physical assault resulting in homicide. However, in both cases, the internal examination revealed superficial, nonfatal injuries. These findings, in the context of the scene characterizations, external body examinations, forensic autopsies, and comprehensive toxicology analyses, underscore the necessity of thorough investigation.[9]

The substructure drug 2C-I, whereof 25I-NBOMe is a derivative, has been reported to cause morbidity in combination with methylenedioxyamphetamine, or MDA,[10] possibly because of its combination of 5-HT2A receptor antagonism with relatively increased agonist activity at the 5-HT2C receptor subtype.[11] The interaction of this drug with these particular receptor subtypes may be significant because they are abundant in neurological tissues.[12]

In contrast to 2C-I, 25I-NBOMe is an extremely potent partial 5-HT2A agonist by affinity and functional phosphatidylinositol hydrolysis assays.[12] Serotonergic hallucinogens may

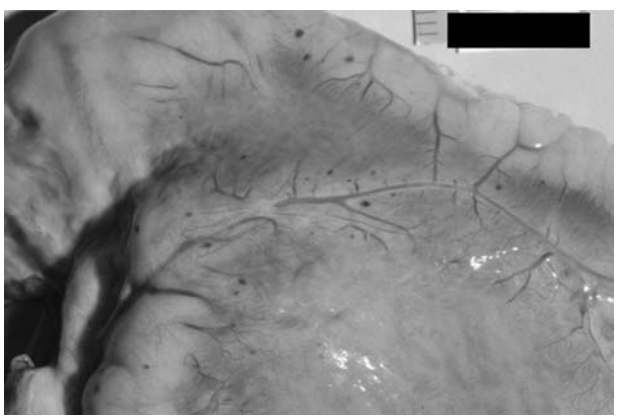

FIGURE 8. Case 1, Petechiae scattered across the epicardial surface.

FIGURE 10. Case 2, White, foamy discharge from the endotracheal tube.

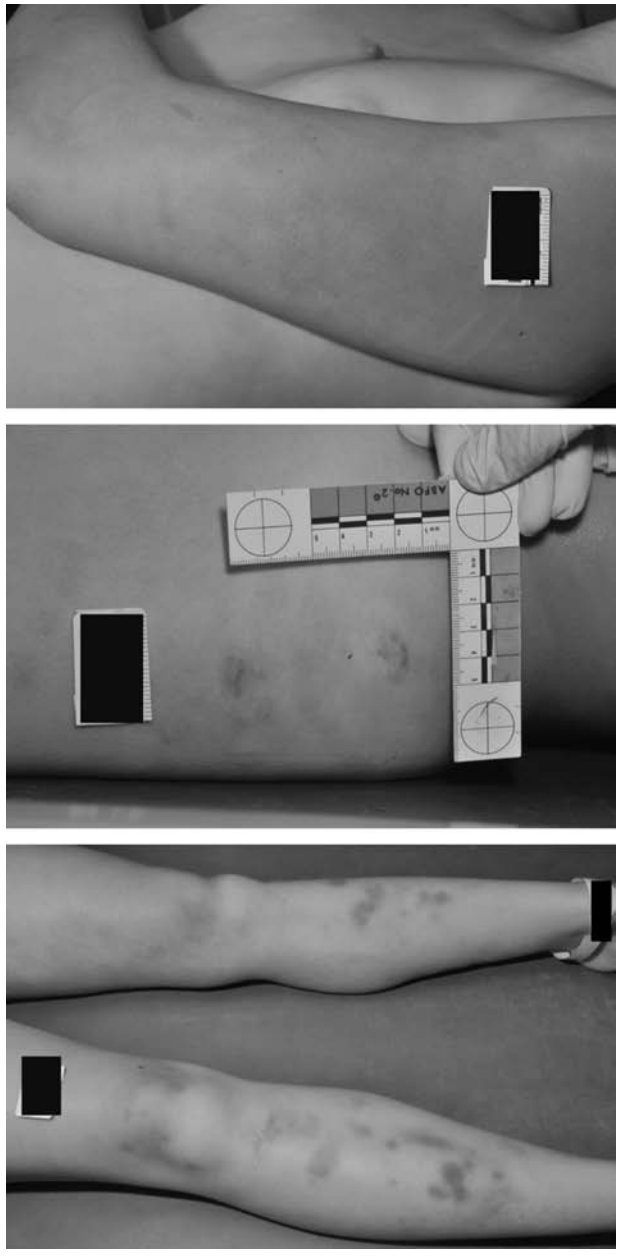

FIGURE 11. Case 2, Numerous abrasions and contusions on the upper extremities, left hip, and shins.

trigger an avalanche of serotonin to precipitate "serotonin syndrome." In addition, 25I-NBOMe may selectively release arachidonic acid to promote prostaglandin formation and inflammation.[13] The cardiovascular and respiratory systems are affected as well; 5-HT2A receptor agonists are sympathoexcitatory, and activation of peripheral 5-HT2 receptors causes smooth muscle vasoconstriction. This in turn mediates a rise in blood pressure and promotes bronchoconstriction,[14–16] likely because of the release of epinephrine and norepinephrine.[17,18]

In a recent report of a patient who survived an interval of 25I-NBOMe intoxication, an 18-year-old male presented to the emergency department with severe agitation and hallucinations after jumping out of a moving car. His pulse rate and blood pressure were 150 to 160 beats per minute and 150 to 170/110 mm Hg, respectively. Physical restraints and intravenous lorazepam were used to manage agitation. His symptoms gradually improved, and his vital signs returned to normal over 48 hours, although he continued to have episodes of aggressiveness. A quantitative assay developed by an analytical toxicology laboratory found that the serum contained 0.76 ng/mL of 25I-NBOMe.[19] This rather low concentration emphasizes the extreme potency of this compound and increased likelihood of overlooking its presence in routine toxicology screens.

25I-NBOMe is not the most potent 5-HT2A receptor agonist, as other iterations have been refined through computer simulations and binding activation experiments.[20,21] New derivatives have been synthesized by replacing the iodine moiety with bromine (25B-NBOMe), chlorine (25C-NBOMe),[22] or small alkyl groups such as methyl (25D-NBOMe), ethyl (25E-NBOMe), or dimethyl (25G-NBOMe).[23] Additional variants result from the substitution of hydroxybenzyl (-NBOH), methylenedioxybenzyl (-NBMD), and fluorobenzyl (-NBF) groups for the methoxybenzyl (-NBOMe) moiety on the opposite end of the molecule.[3,4] Considering the established trends in appearance of research chemicals sold as "legal highs," we anticipate the emergence of many new synthetic compounds and derivatives in the near future. For those investigating deaths possibly involving the recreational use of such compounds, it will be necessary to maintain a level of suspicion that rivals the creativity of those who fabricate these exotic drugs.

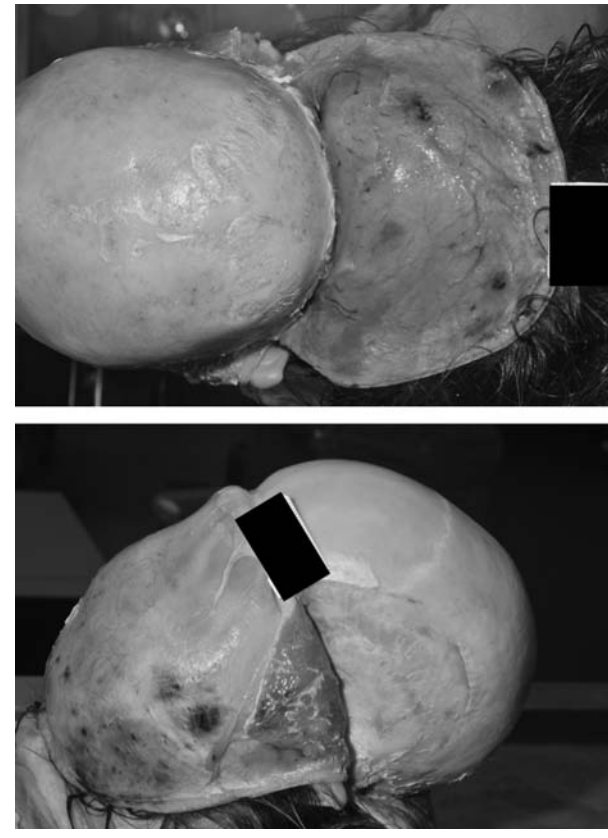

FIGURE 12. Case 2—Subscalpular hemorrhages in the left frontoparietal, right parietal, and right occipital regions.

REFERENCES

1. Fantegrossi WE, Murnane KS, Reissig CJ. The behavioral pharmacology of hallucinogens. *Biochem Pharmacol*. 2008;75:17–33.
2. Braun U, Shulgin AT, Braun G, et al. Synthesis and body distribution of several iodine-131 labeled centrally acting drugs. *J Med Chem*. 1977;20:1543–1546.
3. Braden MR, Parrish JC, Naylor JC, et al. Molecular interaction of serotonin 5-HT2A receptor residues Phe339(6.51) and Phe340(6.52) with superpotent N-benzyl phenethylamine agonists. *Mol Pharmacol*. 2006;70:1956–1964.
4. Ettrup A, Hansen M, Santini MA, et al. Radiosynthesis and in vivo evaluation of a series of substituted 11C-phenethylamines as 5-HT (2A) agonist PET tracers. *Eur J Nucl Med Mol Imaging*. 2011;38:681–693.
5. Ettrup A, Palner M, Gillings N, et al. Radiosynthesis and evaluation of 11C-CIMBI-5 as a 5-HT2A receptor agonist radioligand for PET. *J Nucl Med*. 2010;51:1763–1770.
6. Nichols DE, Frescas SP, Chemel BR, et al. High specific activity tritium-labeled N-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine (INBMeO): a high-affinity 5-HT2A receptor-selective agonist radioligand.*Bioorg Med Chem*. 2008;16:6116–6123.
7. Guale F, Shahreza S, Walterscheid JP, et al. Validation of LC-TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens. *J Anal Toxicol*. 2013;37:17–24.
8. Rao VJ, Wetli CV. The forensic significance of conjunctival petechiae. *Am J Forensic Med Pathol*. 1988;9:32–34.
9. Lorin de la Grandmaison G, Fermanian C, Durigon M. Analysis of discrepancies between external body examination and forensic autopsy. *Am J Forensic Med Pathol*. 2008;29:40–42.
10. Drees JC, Stone JA, Wu AH. Morbidity involving the hallucinogenic designer amines MDA and 2C-I. *J Forensic Sci*. 2009;54:1485–1487.
11. Acuna-Castillo C, Villalobos C, Moya PR, et al. Differences in potency and efficacy of a series of phenylisopropylamine/phenylethylamine pairs at 5-HT(2A) and 5-HT(2C) receptors. *Br J Pharmacol*. 2002;136:510–519.
12. Blaazer AR, Smid P, Kruse CG. Structure-activity relationships of phenylalkylamines as agonist ligands for 5-HT(2A) receptors. *ChemMedChem*. 2008;3:1299–1309.
13. Nichols DE. Hallucinogens. *Pharmacol Ther*. 2004;101:131–181.
14. McCall RB, Clement ME. Role of serotonin1A and serotonin2 receptors in the central regulation of the cardiovascular system. *Pharmacol Rev*. 1994;46:231–243.
15. McCall RB, Patel BN, Harris LT. Effects of serotonin1 and serotonin2 receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. *J Pharmacol Exp Ther*. 1987;242:1152–1159.
16. Ramage AG. Central cardiovascular regulation and 5-hydroxytryptamine receptors. *Brain Res Bull*. 2001;56:425–439.
17. Ramage AG, Villalon CM. 5-hydroxytryptamine and cardiovascular regulation. *Trends Pharmacol Sci*. 2008;29:472–481.
18. Villalon CM, Centurion D. Cardiovascular responses produced by 5-hydroxytriptamine:a pharmacological update on the receptors/mechanisms involved and therapeutic implications. *Naunyn Schmiedebergs Arch Pharmacol*. 2007;376:45–63.
19. Rose SR, Poklis JL, Poklis A. A case of 25I-NBOMe (25-I) intoxication: a new potent 5-HT2A agonist designer drug. *Clin Toxicol (Phila)*. 2013;51:174–177.
20. Silva ME, Heim R, Strasser A, et al. Theoretical studies on the interaction of partial agonists with the 5-HT2A receptor. *J Comput Aided Mol Des*. 2011;25:51–66.
21. Trachsel D, Nichols DE, Kidd S, et al. 4-aryl-substituted 2,5-dimethoxyphenethylamines: synthesis and serotonin 5-HT(2A) receptor affinities. *Chem Biodivers*. 2009;6:692–704.
22. Zuba D, Sekula K, Buczek A. 25C-NBOMe—new potent hallucinogenic substance identified on the drug market. *Forensic Sci Int*. 2012;227:7–14.
23. Zuba D, Sekula K. Analytical characterization of three hallucinogenic N-(2-methoxy)benzyl derivatives of the 2C-series of phenethylamine drugs. *Drug Test Anal*. 2013;5,8:634–645.