REVIEW ARTICLE

Evidence of 2 Populations of Mephedrone Abusers by Hair Testing. Application to 4 Forensic Expertises

Pascal Kintz[a,b,*]

ªX-Pertise Consulting 84 route de Saverne 67205 Oberhausbergen, France; ᵇInstitut de Médecine légale 11 rue Humann 67000 Strasbourg, France

Abstract: ***Background***: New psychoactive substances are conquering the drug scene. Among these substances, cathinone derivatives have been observed since late in the years 2000. At that time, there was evidence of increasing use of the synthetic cathinone mephedrone, particularly amongst clubbers. Although the emergent drugs have an aura of safety, there is an increasing amount of experiences on their secondary effects. Mephedrone is known to induce psychosis.

Method: Given the potential negative effects of mephedrone, the laboratory was asked to test for the drug in hair, a cumulative matrix that can document single, occasional or repetitive abuse of xenobiotics. Mephedrone was tested in hair by GC/MS, using a standard procedure developed for stimulants such as amphetamine or ecstasy.

Results: In the head hair of 24 positive abusers, mephedrone was identified in the range 0.1 to 87 ng/mg, clearly determining 2 populations, one with co-administration of ecstasy and a second without ecstasy. In the first population, mephedrone concentrations were 0.1 to 5 ng/mg; in the second population, mephedrone concentrations were 3 to 87 ng/mg. These findings should help in the understanding the addiction of subjects. In 4 separate forensic cases, mephedrone was identified in hair of abusers, including a rape case (0.54 ng/mg), a fatal car crash (0.38 ng/mg), a fatal drowning (1.21 ng/mg), and a fatal overdose (6.99 ng/mg).

Conclusion: Hair testing for new psychoactive substances appears as a good complement to standard urine analyses. This study confirms the increasing diffusion of new drugs among the forensic population of abusers.

1. INTRODUCTION

New psychoactive substances (NPS) are generally chemical analogues of natural or synthetic psychoactive substances, which, in the many cases are not controlled by the International Conventions on Psychotropic Substances. For the structural similarity to classical drugs and presumptive correspondence in the effects, they may present a relevant public health hazards. An important class of NPS is that of synthetic cathinones, also known as «legal highs», synthesized and used to mimic the effects of psychostimulant drugs such as cathinone, the natural alkaloid from the Khat plant and also cocaine, 3,4 methylenedioxymethamphetamine (MDMA, ecstasy), and methamphetamine. Likely in case of the other NPS, Internet has played and is playing a major role in providing information on how to buy and to use synthetic cathinones with the eventual subjective and side effects [1].

The synthetic cathinone 4-methylmethcathinone (mephedrone or 4-MMC) is undoubtely the most popular derivative of the naturally occurring psychostimulant cathinone. Although already banned in many countries, mephedrone is still freely sold by web sites online and in some stores.

Reports of serious toxicity and deaths following its use have created a substantial scientific, media and public concern. Mephedrone acute intoxication shows clinical features of an acute sympathomimetic toxidrome (*e.g.* hypertension, tachycardia and agitation), similar to the pattern of toxicity of other recreational drugs such as ecstasy and cocaine [2]. The few available data show that mephedrone presents a rather narrow "therapeutic" window that makes its use particularly hazardous. Dosages, which supposedly fall within recreational use limits, can also lead to death when combined with other drugs in certain circumstances [3, 4].

In most abusers, mephedrone causes mood fluctuation in the post-drug recovery period. Parallel variations occur in many psychological and neurocognitive functions, so that consumers undergo various off-drug deficits. Mephedrone

*Address correspondence to this author at the Institut de Médecine légale 11 rue Humann, France; Tel/Fax: + 33 388266431, + 33388 272403;
E-mail: pascal.kintz@wanadoo.fr

affects the hypothalamic-pituitary-adrenal axis, impairs sleep, disrupts homeostasis, and exacerbates psychiatric distress. Neuroimaging studies have revealed altered brain activity patterns in regular users [5].

For all these reasons, it is of importance to document mephedrone abuse. Since the limitations of self-reports drug use are well known, testing for this substance and other illicit drugs in conventional and non conventional biological fluids and matrices is important in many clinical and forensic toxicological situations, both for assessing the cause of the intoxication/death and for evaluation of eventual drug related impairment.

The presence of parent drug and/or a metabolite in a biological specimen can be used to assess both active and passive exposure. The recognized drug testing standard procedure is an initial immunoassay screen, followed by the gas or liquid chromatographic-mass spectrometric confirmation if a non-negative result is obtained in the screening test. The most used biological matrix in clinical and forensic toxicology is urine, followed by blood (as plasma and serum, too). Nevertheless, thanks to the significant advances in sensitive analytical techniques, biological matrices such as hair became an important information tool in drug testing. The main advantage of this sample over traditional one reside in a non invasive, easy to perform collection which may be carried out under close supervision of law enforcement officers to prevent adulteration or substitution. The time window of drug detection is widened to the past weeks, months or even years, thus accounting for long-term exposure. The important value of hair testing for the identification of past chronic or acute use of drugs is increasingly gaining recognition in forensic sciences, in clinical applications and for doping control. The major practical advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance window. Hair analysis may be especially useful when a past history of drug use is difficult or impossible to obtain, such as in situations where collection relates to an event that occurred several weeks or months earlier [6]. The discrimination between a single exposure and long-term use can be documented by multi-sectional hair analysis.

In the past years, several toxicological applications of hair analysis have been published in various situations, including screening of drug addicts (for ethanol, pharmaceuticals and drugs of abuse), child abuse, drug-facilitated crimes, including sexual assaults under the influence of drug(s), doping control in sport, and workplace drug testing.

The purpose of this paper is to present results from hair testing for mephedrone in drug abusers and in four related forensic expertises.

2. HAIR SAMPLES

2.1. Case Reports

Head hair specimens were collected in the context of proving drug abuse, both for forensic or clinical diagnosis of exposure to stimulants (amphetamines, ecstasy, cocaine). In most cases, the hair was collected by an experienced nurse, wrapped in a foil and stored at room temperature until analysis. Hair length, hair colour and any visible cosmetic treatment were indicated upon the official request for testing. The gender of the donors was generally known. There were 112 specimens submitted for analysis, mostly from UK. When possible, a 0 to 3 cm segment was tested. Body hair results are not included in this study.

2.2. Expertise

Expertise 1

A 28 year-old man, plumber, was found dead in the toilets of his home. Tablets and white powders were found in an adjacent room. External body examination revealed the lack of any traumatic injury. Cyanosis was remarkable. During examination, the pathologist collected cardiac blood and hair (light brown, 4 cm). The Prosecutor in charge of the case did not request an autopsy. A comprehensive screening in blood for drugs of abuse identified cocaine (43 ng/mL), benzoylecgonine (1690 ng/mL), norcocaine (6 ng/mL) and mephedrone (5432 ng/mL).

Expertise 2

A 19 year-old girl went to the Police station because she had the feeling of having been raped about 32 hours earlier. She indicated memory loss and non-specific wounds, which were not confirmed by an experienced pathologist. Blood (on EDTA) was collected and toxicological analyses found a recent exposure to both cannabis (THC at 0.6 ng/mL) and mephedrone (31 ng/mL). Ethanol tested negative in blood. Hair collection was requested to document mephedrone abuse (not cannabis). This aimed to demonstrate a possible implication of mephedrone due to its entactogenic effects and its possible anterograde amnesia production (as it is the case with ecstasy). Hair specimen was brown in colour and 21 cm in length and was collected 3 weeks after the alleged offense.

Expertise 3

A 31 year-old man was killed during a car crash against a bridge. After Police request, the subject was submitted to a body examination that revealed numerous injuries. The pathologist collected cardiac blood and hair (black, 3 cm). Ethanol (0.41 g/L) and mephedrone (103 ng/mL) were identified in blood.

Expertise 4

A 18 year-old man was found dead at the surface of a local river. During the autopsy, the pathologist collected femoral blood, urine, bile, gastric content and hair (dark brown, 6 cm). Mephedrone was identified in blood (88 ng/mL), urine (2430 ng/ml) and bile (71 ng/ml). Blood alcohol concentration was 1.06 g/L.

3. ANALYSIS OF MEPHEDRONE IN HAIR

Mephedrone was tested in hair using a slightly modified method published for stimulants [7]. Briefly, the hair was decontaminated by washing twice with 5 mL methylene chloride for 2 min at room temperature. The hair strands were dried and cut into small pieces (<1 mm) with scissors, weighed (30 mg, minimum 20 mg) and incubated overnight at 40 °C in 1 mL phosphate buffer pH 7.4 in the presence of

20 µL of MDMA-d$_5$ (10 mg/L), used as internal standard for mephedrone. For the other stimulants (amphetamine, methamphetamine, MDA, MDMA), the corresponding deuterated standard was used. After alkalinisation with 1 mL of 1 N NaOH, 5 mL of ethyl acetate were added and mixed for 10 min on a horizontal shaker. The samples were then centrifuged and the top organic layer was transferred into glass vials with 100 µL of a mixture of methanol/ hydrochloric acid (99:1, v/v) and dried down under nitrogen. The dry extract was reconstituted in 100 µL of HFBA and 50 µL of ethyl acetate. The eluate was then derivatized for 30 min at 60 °C. The mixture was evaporated to dryness under nitrogen, dissolved in 30 µL of ethyl acetate and transferred to an autosampler vial, where 2 µL were injected onto the chromatographic system.

The GC/MS system consisted of an Agilent 6890 gas chromatograph coupled to a 5973 mass spectrometer. The injector was used in splitless mode at 240°C. The chromatographic separation was achieved on a non- polar capillary column (HP 5 MS, 30 m 0.25 mm i.d. 0.25 mm film thickness). The oven temperature was programmed to increase from 65 (kept for 2 min) to 280°C with helium carrier gas maintained at a constant flow of 1.0 mL/min. Detection was achieved in single ion monitoring (SIM) mode using the detector operating in electron impact mode of ionization at 70 eV. The SIM ions collected were m/z <u>254</u>, 119, 210 for mephedrone and m/z <u>258</u>, 213 for MDMA-d$_5$ (the underlined ions were used for quantification).

In hair, linearity was observed for mephedrone concentrations ranging from 0.1 to 50 ng/mg with a correlation coefficient of 0.999. Within-batch precision at 1 ng/mg was 17.6%. The limit of detection was estimated to be 20 pg/mg, with a S/N ratio of 3. Under the chromatographic conditions used, there was no interference by chemicals or any extractable endogenous materials present in hair.

4. RESULTS AND DISCUSSION

Out of the 112 samples analysed, mephedrone was detected in 24 samples, in concentrations ranging from 0.10 to 86.81 ng/mg, which is consistent with the previous studies, particularly the one of Martin *et al.* [8]. Individual results are presented in Table **1**. MDMA was detected in 16 from 24 specimens. Interestingly in all these latter specimens mephedrone concentration was lower than 5 ng/mg. MDMA was not detected in 8 cases, where mephedrone concentration was always higher than 3 ng/mg. A 25% the hair samples contained cocaine and 16.7% contained amphetamine with one sample also containing methamphetamine.

Obviously, these results suggest the existence of 2 mephedrone-user populations: the first one abusing at the same time ecstasy and/or cocaine, the second one consuming the sole mephedrone. In the first population, mephedrone concentrations were 0.1 to 5 ng/mg; in the second population, mephedrone concentrations were 3 to 87 ng/mg.

The measured mephedrone blood concentrations in the 4 post mortem cases are in the range of what has been published in the forensic literature. The concentrations were potentially fatal in case 1 and indicative of impairment in cases 2, 3 and 4.

Only few papers have been published in the international literature about mephedrone testing in hair. Martin *et al.* published in 2012 the first paper [8]. From 67 specimens collected in the context of narcotics abuse, the authors detected mephedrone in 13 samples using GC/MS, with concentrations ranging from 0.2 to 313.2 ng/mg. They concluded that the developed analytical method was sensitive enough to reveal occasional to regular use of mephedrone. The same year, using LC/MS-MS, Shah *et al.* [9] detected mephedrone in 5 samples out of 154 samples analysed, but succeeded to quantify the drug in only 1, at 21.11 pg/mg and a retrospective study in hair [10] showed that out of 325 hair specimens that originally tested positive for amphetamines or MDMA, 11 (3%) contained also mephedrone. In a recent fatal multidrug intoxication involving mephedrone and cocaine, the authors [11] tested a hair sample which revealed a past exposure to mephedrone (0.25 ng/mg), cocaine (0.78 ng/mg), ketamine (1.90 ng/mg) and MDMA (0.23 ng/mg). Finally, Salomone *et al.* [12] detected mephedrone in 2 hair samples at 50 and 59 pg/mg obtained from proven MDMA and ketamine abusers. Taken together, these papers suggest that many ecstasy abusers may be using other new psychoactive substances and particularly mephedrone.

The simultaneous abuse of ecstasy and new psychoactive substances by young adults has been also observed by other authors using hair as a matrix of evidence [13].

These findings should help in the understanding the possible addiction pattern of psychoactive drugs consumers. Mephedrone and MDMA are among the most popular party drugs with psychoactive effects. Structurally they are amphetamine-type substances with monoamine neurotransmitter enhancing actions. They differed each other in the inhibition of dopamine reuptake into synaptic vesicles from human striatum, with MDMA being 10-fold more potent than mephedrone and their ability to release dopamine from human vesicular monoamine transporter expressing neuroblastoma cells in which MDMA seems to have a stronger effect, giving a molecular explanation to the lower long-term neurotoxicity of mephedrone [14]. One can therefore suppose that abusers will chose the substance which better fits to modify their behaviour. Overall, present data suggest that mephedrone differs not only from MDMA but also from other cathinones in its pharmacological profile and behavioural and neurotoxic effects [15].

Although un-frequent, mephedrone was identified in routine forensic casework. As here reported, during the last 2 years, mephedrone was detected in 4 cases, including a fatal intoxication, an alleged drug-facilitated sexual assault, a traffic accident and a fatal drowning and hair was collected to document past exposure to drugs. Table **2** contains the hair results in these 4 expertises. All hair specimens contained mephedrone and out of the 4 samples analysed, only 1 sample did not contain ecstasy. Police investigations confirmed in all cases that the subjects were nightclub and techno festival attendees.

Unfortunately, no additional data, such as frequency of consumption, were collected in these cases, so there is poor

Table 1.　Distribution of mephedrone concentrations in the 24 positive hair samples.

Case	Gender	Mephedrone (ng/mg)	Other Stimulants (ng/mg)
1	M	0.51	MDMA: 0.87, COC: 1.21
2	M	0.38	MDMA: 2.34, AMPHET: 1.01
3	M	0.10	MDMA: 0.10
4	F	0.59	MDMA: 2.58
5	M	1.87	MDMA: 3.89, COC: 4.55
6		2.59	MDMA: 12.7, AMPHET: 5.01
7	M	0.33	MDMA: 6.36
8	F	4.76	MDMA: 12.52
9	F	2.11	MDMA: 8.04, COC: 2.89
10	M	0.19	MDMA: 3.23
11	M	0.83	MDMA: 4.44, COC: 0.51
12		1.22	MDMA: 8.07
13	M	1.57	MDMA: 9.74
14	M	2.12	MDMA: 4.37, METH: 1.21, AMPHET: 0.28
15	M	1.88	MDMA: 5.41, COC: 10.36
16	F	7.65	
17	M	5.99	COC: 24.31
18		11.87	
19	M	8.27	
20	M	86.81	MDMA: 11.24
21	F	6.72	
22	M	3.04	
23		5.28	
24	M	14.86	COC: 10.19, AMPHET: 0.33

In 4 cases, the gender was unknown COC: cocaine, AMPHET: amphetamine, METH: methamphetamine.

Table 2.　Distribution of mephedrone concentrations in hair from 4 forensic cases.

Case	Forensic Situation	Mephedrone (ng/mg)	Other Stimulants (ng/mg)
1	intoxication	6.99 (whole length, 4 cm)	COC: 14.8
2	sexual assault	0.54 (segment 0-3 cm)	MDMA: 3.14, AMPHET: 2.56
3	car crash	0.38 (whole length, 3 cm)	MDMA: 2.06
4	drowning	1.21 (whole length, 6 cm)	MDMA: 4.02, COC: 2.25

knowledge on the correlation between the frequency and dosage of mephedrone intake and hair concentration. However, mephedrone concentrations in hair are in the same magnitude as the hair MDMA ones [6]. At this time, it appears that there is no controlled study in the scientific literature dealing with administration of a determined dose of mephedrone and analysis of the corresponding hair specimens. Therefore, one does not know what is the expected range of mephedrone concentrations in hair after single exposure to the drug. The concept of minimal detectable dosage in hair is of paramount importance to correctly interpret the results. Without this information and any systematic data from scientific literature, it is easy to dispute any negative hair result.

There are essentially 3 types of problems with testing for drugs of abuse in urine: false-positives, specimen degradation and actions causing adulteration. These problems can be circumvented or even eliminated through hair analysis. It is always possible, where available, to obtain one or more identical hair samples if there is any claim of a specimen

mix-up or breach in the chain of custody. This renders hair analysis basically fail-safe, in contrast to urinalysis, since an identical urine specimen cannot be obtained at a later date. Clearly, hair analysis can thus function as a "safety net" for urine analysis.

Another potential use of hair analysis is to verify intentional or unintentional ingestion of drinks or foods added with drugs. In case of a single use, hair will not test positive in consecutive segments. Its greatest use, however, may be in identifying false-negative drug users, who can abstain from a drug for a few days trying to "beat the urinalysis" since this occurrence will not alter drug concentration in hair. While analysis of urine specimens cannot distinguish between chronic use or single exposure, hair analysis can make this distinction.

By providing information on past repeated exposure to drugs over time, hair analysis may be useful in verifying self-reported declarations in any situation in which a history of past rather than recent drug use is required. By hair testing, a drug user is not able to hide substance abuse since the window of detection is often wide, *i.e.* months [6].

Furthermore, to obtain a retrospective calendar of an individual's drug use segmental analysis is performed which consists on taking a length of hair and cutting it into sections to measure the presence of drug in that specific segment corresponding to a shorter periods of time. The hair must be cut as close as possible to the scalp and particular care is also required to ensure that the individual hairs in the cut-off tuft retain the position they originally had beside one another.

CONCLUSION

Identification and quantitation of mephedrone in human hair contribute to new information about the pattern of use of this new psychoactive substance. Mephedrone intoxications and fatalities have been described in the more recent period [3, 16]. Hair analysis can be of great value in these cases, as analysis of the subjects' hair can establish the regular abuse of mephedrone. The major advantage of hair testing compared with blood or urine testing is a larger detection window (weeks to months), depending on the length of the hair shaft. Hair is a unique material for retrospective investigation of chronic drug use. It is useful to diagnose an history of drug abuse or relapse and for monitoring patients in maintenance programs. It can demonstrate addictive behaviours among specific populations for which it is extremely difficult to obtain positive blood and/or urine samples. Hair testing for drugs of abuse can also validate or invalidate self-reported drug use, especially in situations where the statements are cautious.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Karila, L.; Megarbane, B.; Cottencin, O.; Lejoyeux, M. Synthetic cathinones: a new public health problem. *Curr. Neuropharmacol.,* **2015**, *13*(1), 12-20. [http://dx.doi.org/10.2174/1570159X13666141210224137] [PMID: 26074740]

[2]   Wood, D.M.; Davies, S.; Greene, S.L.; Button, J.; Holt, D.W.; Ramsey, J.; Dargan, P.I. Case series of individuals with analytically confirmed acute mephedrone toxicity. *Clin. Toxicol. (Phila.),* **2010**, *48*(9), 924-927. [http://dx.doi.org/10.3109/15563650.2010.531021] [PMID: 21171849]

[3]   Busardò, F.P.; Kyriakou, C.; Napoletano, S.; Marinelli, E.; Zaami, S. Mephedrone related fatalities: a review. *Eur. Rev. Med. Pharmacol. Sci.,* **2015**, *19*(19), 3777-3790. [PMID: 26502870]

[4]   Chung, H.; Lee, J.; Kim, E. Trends of novel psychoactive substances (NPSs) and their fatal cases. *Forensic Toxicol.,* **2016**, *34*, 1-11. [http://dx.doi.org/10.1007/s11419-015-0286-5]

[5]   Parrott, A.C. Why all stimulant drugs are damaging to recreational users: an empirical overview and psychobiological explanation. *Hum. Psychopharmacol.,* **2015**, *30*(4), 213-224. [http://dx.doi.org/10.1002/hup.2468] [PMID: 26216554]

[6]   Kintz, P. Drugs in hair. In: *Encyclopedia of Forensic Sciences,* 2nd ed; Siegel, J.A.; Saukko, P.J., Eds.; Academic Press: Waltham, **2013**: pp. 360-364. [http://dx.doi.org/10.1016/B978-0-12-382165-2.00305-6]

[7]   Sachs, H.; Kintz, P. Testing for drugs in hair. Critical review of chromatographic procedures since 1992. *J. Chromatogr. Biomed. Sci. Appl.,* **1998**, *713*(1), 147-161. [http://dx.doi.org/10.1016/S0378-4347(98)00168-6] [PMID: 9700557]

[8]   Martin, M.; Muller, J-F.; Turner, K.; Duez, M.; Cirimele, V. Evidence of mephedrone chronic abuse through hair analysis using GC/MS. *Forensic Sci. Int.,* **2012**, *218*(1-3), 44-48. [http://dx.doi.org/10.1016/j.forsciint.2011.10.016] [PMID: 22041623]

[9]   Shah, S.A.; Deshmukh, N.I.; Barker, J.; Petróczi, A.; Cross, P.; Archer, R.; Naughton, D.P. Quantitative analysis of mephedrone using liquid chromatography tandem mass spectroscopy: application to human hair. *J. Pharm. Biomed. Anal.,* **2012**, *61*, 64-69. [http://dx.doi.org/10.1016/j.jpba.2011.11.026] [PMID: 22209483]

[10] Rust, K.Y.; Baumgartner, M.R.; Dally, A.M.; Kraemer, T. Prevalence of new psychoactive substances: A retrospective study in hair. *Drug Test Anal.,* **2012**, *4*(6), 402-408. [http://dx.doi.org/10.1002/dta.1338] [PMID: 22522922]

[11] Gerace, E.; Petrarulo, M.; Bison, F.; Salomone, A.; Vincenti, M. Toxicological findings in a fatal multidrug intoxication involving mephedrone. *Forensic Sci. Int.,* **2014**, *243*, 68-73. [http://dx.doi.org/10.1016/j.forsciint.2014.04.038] [PMID: 24846124]

[12] Salomone, A.; Gazzilli, G.; Di Corcia, D.; Gerace, E.; Vincenti, M. Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real samples. *Anal. Bioanal. Chem.,* **2016**, *408*, 2035-2042. [PMID: 26680593]

[13] Palamar, J.J.; Salomone, A.; Vincenti, M.; Cleland, C.M. Detection of bath salts and other novel psychoactive substances in hair samples of ecstasy/MDMA/"Molly3 users. *Drug Alcohol Depend.,* **2016**. pii: S0376-8716(16)00057-0. doi: 10.1016/j.drugalcdep.2016.02.001

[14] Pifl, C.; Reither, H.; Hornykiewicz, O. The profile of mephedrone on human monoamine transporters differs from 3,4-methylenedioxymethamphetamine primarily by lower potency at the vesicular monoamine transporter. *Eur. J. Pharmacol.,* **2015**, *755*, 119-126. [http://dx.doi.org/10.1016/j.ejphar.2015.03.004] [PMID: 25771452]

[15] Green, A.R.; King, M.V.; Shortall, S.E.; Fone, K.C. The preclinical pharmacology of mephedrone; not just MDMA by another name. *Br. J. Pharmacol.,* **2014**, *171*(9), 2251-2268. [http://dx.doi.org/10.1111/bph.12628] [PMID: 24654568]

[16] Busardò, F.P.; Kyriakou, C.; Tittarelli, R.; Mannocchi, G.; Pantano, F.; Santurro, A.; Zaami, S.; Baglìo, G. Assessment of the stability of mephedrone in ante-mortem and post-mortem blood specimens. *Forensic Sci. Int.,* **2015**, *256*, 28-37. [http://dx.doi.org/10.1016/j.forsciint.2015.07.021] [PMID: 26295910]