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Mephedrone related fatalities: a review

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Abstract. – OBJECTIVE: Synthetic cathinones are an emerging class of designer drugs abused of due to their psychostimulant and hallucinogenic effects, similar to those of cocaine, methylenedioxymethamphetamine (MDMA), amphetamines and methamphetamines. Mephedrone is a cathinone analogue (4-methyl aromatic analogue of methcathinone) that was reported to be implicated in several fatalities in the media across Europe, but only a few have actually resulted in mephedrone cited as the cause of death. In this paper, we aim to systematically review analytically confirmed cases of mephedrone-related fatalities.

MATERIALS AND METHODS: Relevant scientific articles were identified from Medline, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE and Google Scholar, through May 2015 using the following keywords: "Mephedrone", "fatal intoxication," "fatalities", "acute intoxication" and "death".

RESULTS: In total, 10 citations met the criteria for inclusion, representing 18 fatal cases with analytically confirmed mephedrone in biological sample/s of the deceased. The death was attributed to mephedrone intoxication in 9 cases (range of post-mortem blood mephedrone concentration: 1.33-22 mg/L), whereas multiple drug toxicity, involving mephedrone was cited as cause of death in 6 cases (range of post-mortem blood mephedrone concentration: 0.04-1.3 mg/L).

CONCLUSIONS: Data suggest that the abuse of mephedrone remains to be a public health issue. Mephedrone appears to have a rather narrow therapeutic window that makes its use dangerous. Dosages which supposedly fall within recreational use limits could also lead to death when combined with other drugs in certain circumstances. Forensic Toxicology laboratories must assess their testing procedures to ensure they can achieve both an appropriate screening regime and targeted quantitative analysis for the detection of mephedrone in various biological matrices.

Key Words:
Mephedrone, Fatalities, Acute intoxication, Death.

Introduction

In recent years, a large number of new, originally non-controlled designer drugs have emerged on the legal drug market, also known as bath salts, legal highs[1,2], pond cleaner, plant food or fertilizers, insect repellent, or vacuum fresheners. This recent phenomenon of new psychoactive substances (NPS), is a global issue with the ever-growing number of new substances available on the illicit drug market. In many countries, such substances are rarely included in drug control legislation and may even fall outside the generic legislative systems, due to the fact that these drugs are predominantly synthetic derivatives and analogues of existing controlled drugs, pharmaceutical products, previously research chemicals or naturally occurring compounds[3,4]. These drugs are available in packets that contain milligram to gram quantities. They are most commonly sold as bath salts with the disclaimer, "Not For Human Consumption"[5-7].

Mephedrone [4-methylmethcathinone; 4-methylephedrone; 4-MMC; UPAC: 2-(methylamino)-1-(4-methylphenyl)propan-1-one] belongs to the category of designer drugs and it is structurally similar to cathinone, which is the principal active constituent of the Khat plant (Catha edulis)[8]; also known as natural amphetamine due to its stimulant properties[9,10]. Synthesized as a cathinone analogue[11] (4-methyl aromatic analogue of methcathinone), the structural similarities between mephedrone and methcathinone are shown in Figure 1.

Mephedrone was first synthesized in 1929 as a ring-substituted cathinone and its structure is closely related to that of the phenethylamine family[12]. Mephedrone induces stimulant psychoactive effects similar to those produced by amphetamines, methamphetamines, 3,4-methylenedioxy-N-methylamphetamine (MDMA) and

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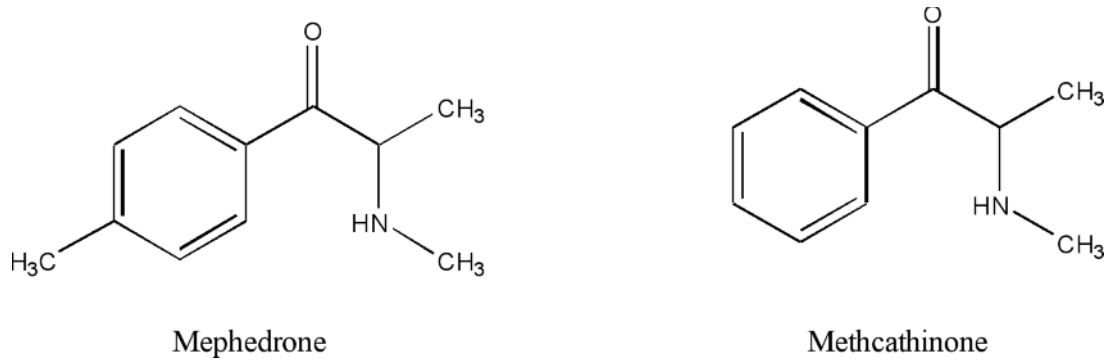

Figure 1. Structures of mephedrone and methcathinone.

cocaine, including hallucinations, psychosis and distinct psychotropic effects. Due to its properties, mephedrone is becoming increasingly popular as a recreational drug, mainly among youths (young clubbers), though its use is also reported in other age groups, including young adults, mid-to-late adolescents and older adults[13-15].

Mephedrone has become available from several sources, such as street drug dealers, head shops[16] marketed as a "legal high", internet suppliers marketed as a research chemical or plant food[17], various convenience stores, truck shops, gas stations and tattoo parlours[18]. Arguably the available information, accessibility and the promotion through aggressive web-based marketing, has led to a marked increase of its use, therefore making it a significant public health threat both in Europe and US[19-21].

It is sold as a white or slightly yellowish powder or fine crystals under different street names, including "4-MMC", "MMCAT", "bubbles", "Crab", "meow meow", and "M-Cat"[17,19,22,23]. Less frequently it is marketed as tablets or capsules with different colours, shapes and thickness, either with or without a logo. Sometimes mephedrone is sold as either cocaine or ecstasy while cut-agents such as paracetamol, caffeine, amphetamine, ketamine and cocaine may be found in mephedrone[19,24].

The most common routes for recreational use are inhalation (snorting) and oral ingestion, but due to its high water solubility, mephedrone is also injected, taken by rectal insertion and intramuscular injection[12,25]. Co-administration of mephedrone with alcohol and/or other controlled substances like GHB, heroin and MDMA has been reported[17,26-28].

Reports from users at hospital toxicology departments illustrate that mephedrone is taken in staggered doses[13]. Mephedrone tolerance may be quickly developed, and therefore, users tend to consume higher doses more frequently[29]. Mephedrone dosages of 200 mg or more have been self-reported, with some users reporting redosing to prolong the euphoric state, leading to a total dosage of 1-2 g administered in a session[29].

Mephedrone abuse has serious side effects, including increasing heart rate, chest pain, change in body temperature (sweating chills), agitation, headache hypertension, insomnia, irritability, minor amnesia dilated pupils, seizure, nausea, vomiting and dizziness[12,19,29,30]. The effects induced by ring substituted cathinones include feelings of stimulation (alertness, rushing), empathy (love, openness, closeness, well-being and sociability), euphoria, awareness of senses and appreciation of music[29,31].

In April 2010 mephedrone and structurally related substances were classified as Class B substances in the UK under the Misuse of Drugs Act. Legislation[32]. Moreover, in response to this raising epidemic of drug abuse, in October of 2011, the Drug Enforcement Administration (DEA) issued a temporarily Schedule I for the more commonly encountered drugs of cathinone class: mephedrone, methylone, and 3,4-methylenedioxypyrovalerone (MDPV)[33]. This drug has also been classified in some other countries as a measure for the control of its availability. However, after regulatory measures restricting possession, sale, and manufacture of synthetic cathinones passed in the UK, the number of users who purchased the drug from dealers increased considerably and at the same time its price became almost twice as high as before legislation[32].

Although, a number of cases of acute and lethal intoxication have been reported in the literature, little is still known of the correlation

between its blood concentration and its effects. Moreover, most routine drug screening procedures do not include synthetic cathinones in general and mephedrone in particular, thus, preventing clear knowledge of the real consumption of these new drugs within the population and the correlation between the abuse of NPS and road and work accidents and fatal intoxication cases[34]. In this review, we aim to systematically review analytically confirmed cases of mephedrone-related fatalities.

Materials and Methods

Relevant scientific articles were identified from Medline, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE and Google Scholar, through May 2015 using the following keywords: "Mephedrone", "fatal intoxication," "fatalities", "acute intoxication" and "death". The main key word "Mephedrone" was individually searched in association to each of the others. The 22 sources found after the initial screening, in order to exclude duplicate sources and retrospective studies, were selected according to the "inclusion criteria". A comprehensive flow diagram with inclusion criteria is reported in Figure 2. The inclusion criteria covered those citations that:

1) They were written in English;
2) Described human administration of mephedrone;
3) Analytically confirmed the presence of mephedrone;
4) Death was attributed to mephedrone either alone or in combination with other substances.

Results

A total of 22 citations were identified, but only 10 met the inclusion criteria[12,17,28,29,35-40]. In addition, in one of the citations which reported 4 cases of mephedrone fatalities, 2 cases were excluded since the cause of death was unknown in one of the cases and stab wound of the abdomen was cited as cause of death in the other one. However, mephedrone was detected in blood samples in both cases at concentration of 5.7 and 1.2 mg/L, respectively[35]. Moreover, in another citation which reported 12 fatal cases after mephedrone consumption, only 4 cases were included in this review, since in the rest of the cases death was at-

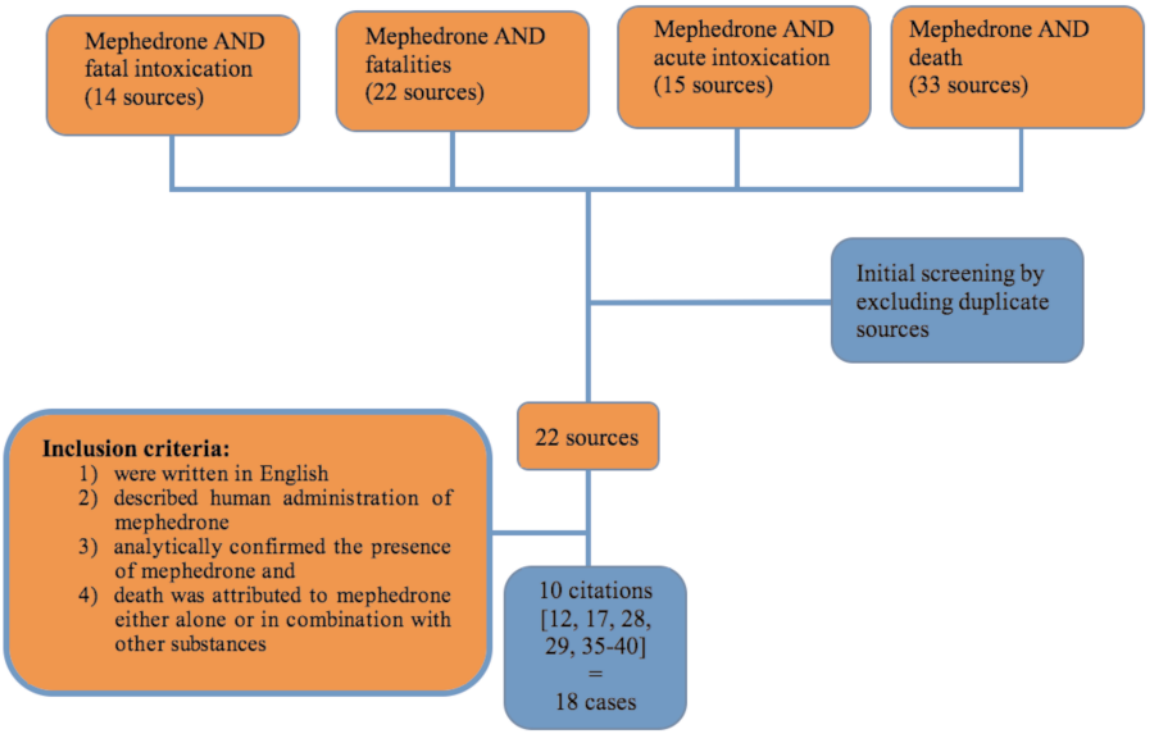

Figure 2. Flow diagram with inclusion criteria for the selection of sources for the purpose of the review.

tributed to mechanical suicide[28]. Lastly, one case was excluded from another source since the death was attributed to 4-MEC[38]. In total, 10 citations met the criteria for inclusion, representing 18 fatal cases with analytically confirmed mephedrone in biological sample/s of the deceased. The extracted data are summarized in the Table I and Table II.

Of the deceased 14 were men, 2 were women and in two cases gender was not reported, with an average age of 27.8 (range: 17-55). Death was attributed to mephedrone intoxication in 9 cases (range of post-mortem blood mephedrone concentration: 1.33-22 mg/L), whereas multiple drug toxicity, involving mephedrone was cited as cause of death in 6 cases (range of post-mortem blood mephedrone concentration: 0.04-1.3 mg/L). However, it is unfortunate that some studies do not give a sampling site from where the post-mortem blood was collected.

The drugs involved in multidrug toxicity cases were heroin, MDMA, methadone, cocaine, alcohol, GHB, etc. In one case, mephedrone was not the primary cause of death but a fatal vehicular collision; though mephedrone may have affected the driving performance[36]. In another case, death was not attributed solely to mephedrone but to the adverse effects of this drug, with cardiac fibrosis and atherosclerotic coronary artery disease as a contributing factor[36]. In the remaining case, a 36-year old man, injured himself severely by smashing windows in a rage of fury and died despite resuscitation attempts. Toxicological analysis showed a high femoral blood mephedrone concentration (5.1 mg/L) and traces of cocaine, MDMA and oxazepam. His death was attributed to fatal oral intake of mephedrone, which probably led to a state of excited delirium successively aggravated by blood loss from multiple wounds[37].

Discussion

The abuse of mephedrone remains to be a public health issue even after its classification. The findings of Winstock et al[32] suggest that drug classification has had a limited effect on controlling its availability and use; in fact in a survey conducted by the same research group it was found that 63% of the responders had continued to use mephedrone after the introduction of the legislation. Before the legislation, users generally had access to mephedrone via the internet, but now they buy it from street dealers, on average at double the price.

Cosbey et al[28] reported 32 non-fatal impaired driving cases where mephedrone was found either alone or in combination with other drugs and/or alcohol. In 9 cases mephedrone was the only drug found at blood concentrations ranging from 0.08 to 0.66 mg/L while in the other 23 cases, mephedrone was detected together with other drugs and the blood concentration was in the interval of 0.01-0.74 mg/L. The data presented in this study suggest that recreational use of mephedrone can produce blood concentrations as high as 0.74 mg/L, although the most common value encountered is likely to lie between 0.2 and 0.3 mg/L. The most common symptoms observed in impaired drivers were agitation, hyperactivity, dilated pupils, glazed eyes and slurred speech. Moreover, authors concluded that on the basis of the evidence presented, fatality due to mephedrone alone is likely to be associated with blood mephedrone concentrations greater than 2.0 mg/L. In the present review the mephedrone concentrations reported in post-mortem blood, where death was attributed to multidrug toxicity, ranged from 0.04 to 1.3 mg/L. The latest ascertainment suggests that mephedrone concentrations which supposedly fall within recreational use limits could also lead to death when combined with other drugs in certain circumstances.

Furthermore, Wikstrom et al[39] highlight, after reporting two accidental deaths attributed to mephedrone intoxication, that the blood concentrations are close to those found in the living, a fact that suggests a rather narrow therapeutic window and emphasizes the dangers associated with the use of this substance.

Wood et al[13] reported a non-fatal intoxication, in which plasma mephedrone concentration was 0.15 mg/L. The 22-year-old man developed features of sympathomimetic toxicity shortly after the mephedrone injection, including palpitations, "blurred tunnel vision," sweating, chest pressure and felt generally unwell[13]. Mephedrone intoxications that required hospitalization were reported after doses of 0.1-7.0 g[29]. However, it is impossible to determine a safe mephedrone dose, since negative side effects may develop in association with any dosage taken. In addition, similar dosages may have dramatically different consequences in different individuals[41]. Dickson et al[17] reported an accidental death caused by the combined use of mephedrone and heroin. The room-

Table I. Mephedrone concentrations in different biological matrices and other drugs and/or alcohol in mephedrone-related deaths.

Study/year	Number of cases	Age, gender	Method of quantification of mephedrone	Mephedrone (mg/L)	Other toxicological findings (mg/L)	Cause of death
Dickson et al[17] 2010	1	22 M	GC–MS	Blood: 0.5 Urine: 198	Blood: morphine:0.06 Urine: doxylamine:1.99 6-acetylmorphine:0.53 Morphine: 27.13 Codeine: 1.86	- multiple-drug toxicity - manner of death: accidental
Torrance and Cooper[35] 2010	2		GC–MS	Case 1: Blood: 22 Urine: positive	Case 1: Blood: diazepam and nordiazepam: < 0.1 amphetamine: 0.34	Case 1: mephedrone intoxication
				Case 2: Blood: 3.3 Urine: >0.5 Hair: 4.2 and 4.7 ng/mg	Case 2: Urine: low concentrations of benzoylecgonine and 11-nor-delta-9- tetrahydrocannabinol-9-carboxylic acid	Case 2: mephedrone intoxication
Maskell et al[36] 2011	4	49 F	HPLC-DAD	Case 1: Femoral venous Blood: 0.98 Urine: positive Stomach content: positive	Case 1: Blood: Atropine, Naloxone [from resuscitation attempts] Urine and stomach contents: low levels of paracetamol	Case 1: Adverse effects of mephedrone with atherosclerotic coronary artery disease and myocardial fibrosis being contributing factors.
		19 M	HPLC-DAD	Case 2: femoral venous Blood: 2.24 Urine: positive	Case 2: Blood: Atropine 3-TFMPP Urine: Acetone Ethanol: 15 mg/dL	Case 2: Mephedrone intoxication

Table continued

Table I. *(Continued).* Mephedrone concentrations in different biological matrices and other drugs and/or alcohol in mephedrone-related deaths.

Study/year	Number of cases	Age, gender	Method of quantification of mephedrone	Mephedrone (mg/L)	Other toxicological findings (mg/L)	Cause of death
		55F	HPLC-DAD	Case 3: femoral venous Blood: 0.13	Case 3: Blood: Diazepam Nordiazepa Olanzepine Chlorpromazine metabolite Methadone: 0.3 Urine: methadone, EDDP, procyclidine, putrefactants (Prescribed drugs)	Case 3: Multiple drug toxicity death [methadone and mephedrone]
		17 M	HPLC-DAD	Case 4: fluoride oxalate preserved femoral venous blood: 0.23 Urine: positive Stomach contents: positive		Case 4: Fatal vehicular collision (mephedrone may have affected the driving performance)
Lusthof et al[37] 2011	1	36M	HPLC–MS/MS	Femoral blood: 5.1 Urine: 186 Stomach contents: 1.04 g/L	Femoral blood: Cocaine: 0.0071 Benzoylecgonine: 0.17 Methylecgonine: 0.042 MDMA: 0.011 Oxazepam: < 0.010 Midazolam: 0.0064 Blood from the heart: Metanephrine: traces may be present Atropine: traces may be present	Fatal intake of mephedrone, which probably led to a state of excited delirium. This was aggravated by blood loss from multiple wounds.

Table continued

Table I. *(Continued).* Mephedrone concentrations in different biological matrices and other drugs and/or alcohol in mephedrone-related deaths.

Study/year	Number of cases	Age, gender	Method of quantification of mephedrone	Mephedrone (mg/L)	Other toxicological findings (mg/L)	Cause of death
Rojek et al[38] 2014	1	29M	LC-MS/MS	Femoral blood: mephedrone: 1.3	Femoral blood: Methcathinone: 0.210 Ethanol: 2.8%	Acute circulatory and respiratory failure resulting from complex intoxication with cathinone derivatives, including mephedrone and methcathinone, in an alcohol-addicted man who was under the influence at the time of death.
Gerace et al[12] 2014	1	25M	GC-MS	Heart Blood: 1.33 Urine: 144 Gastric content: 4.52 Bile 1.29 Lung: 0.79 mg/kg Brain: 0.89 mg/kg Hair: 0.25 ng/mg	Heart blood: Cocaine 18 ng/mL Cocaethylene 18 ng/mL Ethanol 0.13 g/L Lidocaine: positive Phenacetin: positive Paracetamol: positive Levamisole: positive Urine: Ethanol: 0.43 g/L Benzoylecgonine: 34.5 Cocaine: 6.97 Cocaethylene: 3.10 Lidocaine: positive Paracetamol: positive Levamisole: positive Gastric content: Ethanol: 0.23g/L Hair: Cocaine: 0.78 ng/mg Benzoylecgonine: 0.49 ng/mg Ketamine: 1.90 ng/mg MDMA: 0.23 ng/mg	Mephedrone intoxication
Wikström et al[39] 2010	2	23M	GC-MS	Case 1: Antemortem blood: 13.2 μg/g Postmortem femoral blood: 8.4 μg/g	Case 1: Blood: Diazepam: 0.05 μg/g Midazolam: 0.02 μg/g Lidocaine: 0.6 μg/g	Case 1: Accidental mephedrone poisoning

Table continued

Table I. *(Continued).* Mephedrone concentrations in different biological matrices and other drugs and/or alcohol in mephedrone-related deaths.

Study/year	Number of cases	Age, gender	Method of quantification of mephedrone	Mephedrone (mg/L)	Other toxicological findings (mg/L)	Cause of death
		19M	GC-MS LC-MS-MS [for hair analysis]	Case 2: Femoral blood: 9.6 μg/g Hair: 1st segment: 37 ng/mg 2nd segment: 33 ng/mg 3rd segment: 29 ng/mg 4th segment: 29 ng/mg 5th segment: 36 ng/mg	Case 2: Urine: acetone: 0.037 g/dL	Case 2: Accidental mephedrone poisoning
Cosbey et al[28] 2013	4	17M	LC-MS	Case 1: Blood: 0.22	Case 1: BZP [benzylpiperazine]: 1.83 mg/L TFMPP [trifluoromethylphenylpiperazine]: 0.14 Dextromethorphan: 0.96 11-nor-delta-9-tetrahydrocannabinolic acid: 0.13	Case 1: Mixed drug toxicity
		20M	LC-MS	Case 2: Blood: 2.10	Case 2: Blood: alcohol:19 mg/dL Urine:alcohol:49 mg/dL	Case 2: 'Poisoning by mephedrone'.
		20M	LC-MS	Case 3: Blood: 0.04	Case 3: MDMA: 0.51 Diazepam: 1.13 Codeine: 0.04 11-nor-delta-9-tetrahydrocannabinolic acid	Case 3: Poisoning by MDMA and mephedrone
		21M	LC-MS	Case 4: Ante-mortem whole blood: S1: (06:00 hrs) 1.94 S2: (08:00 hrs) 1.69 S3: (09:00 hrs) 0.94 S4: (09:30 hrs) 0.85	Case 4: Blood: (sample 1) TFMPP: 0.02 Diazepam: 0.11	Case 4: Mephedrone toxicity
Adamowicz et al[29] 2013	1	30M	LC-MS-MS	Blood: 5.5 Vitreous humour: 7.1	-	Mephedrone intoxication
Aromatario et al [40] 2012	1	43M	GC-MS	Blood: 0.5 Urine: 14.8 Bile: 1.9 Gastric content: 38	Heart blood: Ethanol: <0.1 g/l. GHB: 288 Urine: GHB: 2900 Bile: GHB: 987 Gastric content: GHB: 224	Multi drug intoxication-a direct toxic effect of GHB and mephedrone

Table II. Circumstances of death and autopsy findings in mephedrone-related deaths.

Study/year	Age, gender	Route of administration	Circumstances of death	Autopsy findings
Dickson et al[17] 2010	22 M	- Insufflation - Intravenous - Oral	- Found unresponsive in his living quarters - Transported to the hospital where he died. - Drug paraphernalia, syringes, suspected heroin, and other controlled substances including 2C-T-7 (2,5-dimethoxy-4-(n)-propylthiophenethylamine) were found in deceased living quarters - "Black Tar" heroin, mephedrone, and 2C-T-7 in decedent's roommate possession	- Multiple needle marks along the decedent's lower legs and ankles. - No other signs of physical trauma
Torrance and Cooper[35] 2010			Case 1&2: suspected use of mephedrone	
Maskell et al[36] 2011	Case 1: 49 F	Case 1: - Alcohol consumption - Cannabis smoking - Insufflation of 0.5 g of mephedrone	Case 1: - Intake of mephedrone - 2-4 h after the intake she complained of a sore chest and vomited - Collapsed - Resuscitation at the scene - Transported to hospital - Further life-saving attempts - Pronounced dead	Case 1: Old atherosclerotic occlusion of the proximal anterior descending coronary artery and a 15-mm diameter area of myocardial fibrosis within the anterior left ventricular wall near the apex
	Case 2: 19 M	Case 2: Alcohol, ecstasy and mephedrone co-administration	Case 2: - Mephedrone intake - Started to shake and twitch, and was described as being sweaty and acting strangely few hours later - "His eyes were rolling and he was choking", according to witnesses - Resuscitation efforts on the arrival of the emergency services and en route to hospital. - Cardio-respiratory arrest in the ambulance - Further life-saving attempts - Pronounced dead at the hospital.	Case 2: No natural disease or trauma that could have contributed to his death.
	Case 3: 55 F	Case 3: Ingestion of unknown drugs and alcohol	Case 3: - Found unresponsive at home - History of psychiatric problems and drug abuse. - Resuscitation attempts - Pronounced dead	Case 3: Focal single artery coronary atherosclerosis with no other natural disease or trauma

Table continued

Table II. *(Continued).* Circumstances of death and autopsy findings in mephedrone-related deaths.

Study/year	Age, gender	Route of administration	Circumstances of death	Autopsy findings
	Case 4: 17 M		Case 4: - Car accident (While driving on the on the wrong side of a straight road, he collided head-on with an oncoming car) - Pronounced dead at the scene	Case 4: Multiple blunt force injuries and no natural disease
Lusthof et al[37] 2011	36 M	Oral	- Injured himself severely by smashing windows in a rage of fury - Arrested by police. - Resuscitation efforts - Pronounced dead - Tablets containing mephedrone were found in the house of the deceased	- Several superficial veins and a tendon of the hand were cut. - Superficial skin lacerations, bruises and minor brain swelling - Lung oedema
Rojek et al[38] 2014	29 M		- Found in the street leaning against a local garage. - His lips, ears and face were bluish and he was foaming at the mouth. - Ambulance was called - Resuscitation efforts - Death was pronounced. His case history indicated that the man abused alcohol and addictive substances.	- Small skin abrasions on the posterior surface of the body, splenomegaly, considerable venous congestion in the internal organs, fatty degeneration of the hepatic parenchyma, and pulmonary oedema. - Cardiac muscle - Vascular wall thickening, proliferation of perivascular connective tissue walls, profound congestion - Multi-visceral congestion
Gerace et al[12] 2014	25 M	Oral	- Found dead in the apartment of a friend after a night spent in several local clubs with a friend drinking alcohol and consuming cocaine. - Fragment of a blue diamond-shaped pill was found in his trousers' pocket - Frequent consumer of new designer drugs	- General pulmonary oedema and multi-visceral congestion. - No evidence of natural disease or trauma.

Table continued

Table II. *(Continued).* Circumstances of death and autopsy findings in mephedrone-related deaths.

Study/year	Age, gender	Route of administration	Circumstances of death	Autopsy findings
Wikström et al[39] 2010	Case 1: 23M	Case 1: Oral	Case 1: - Ingestion of a recreational drug party - Became sick - Upon arrival to the emergency department at the hospital, he was unconscious with a body temperature of 42°C (107.6°F). - Resuscitative efforts - Suffered complete organ failure - Pronounced dead the following day, 16 h after admission. At the hospital, a blood sample was collected and submitted for drug screening	Case 1: Pulmonary edema, pulmonary congestion
	Case 2: 19M		Case 2: - found unconscious at home in an empty bathtub. - during ambulance transport, he developed seizures and became lifeless. - upon arrival at the hospital, he had stopped breathing and heart activity was negligible. - resuscitation attempts - pronounced dead	Case 2: Pulmonary edema, pulmonary congestion
Cosbey et al[28] 2013	Case 1: 17M	Case 1: Snorting during weekend Recent oral administration	Case 1: - 'snorting mephedrone' most of the weekend - recently ingested some powdered mephedrone. - after getting into difficulty in the early hours, an ambulance was called - no pulse upon arrival at hospital - declared dead shortly later	Case 1: - pulmonary oedema, - no evidence of pre-existing natural disease or trauma.
	Case 2: 20M		Case 2: - collapsed at a party. - Suspicions were that he had taken drugs.	Case 2: - evidence of aspiration, but this was believed to be a terminal event. - no evidence of pre-existing natural disease or trauma.
	Case 3: 20M		Case 3: - found dead in bed. - he was thought to have been drinking or taking drugs recently.	Case 3: - pulmonary oedema

Table continued

Table II. *(Continued).* Circumstances of death and autopsy findings in mephedrone-related deaths.

Study/year	Age, gender	Route of administration	Circumstances of death	Autopsy findings
	Case 4: 21M		Case 4: - suspected drug use - became ill - hospitalized - died in Intensive Care - ante-mortem samples were obtained	Case 4: - small amounts of blood-stained fluid in the stomach and abdomen - no evidence of pre-existing natural disease or trauma
Adamowicz et al[29] 2013	30M		- found in a critical state in a staircase. - resuscitation attempts - the man died at the scene. - 8 small plastic bags, each containing 1 g of white powder, were found in the pocket of his jacket.	- brain stem failure and lung injury.
Aromatario et al[40] 2012	43M		- died during a drug-fuelled party - found after an unknown period of unconsciousness - A friend of him reported that they had been offered a drink named "G", which they refused, but both had drunk a glass of water that tasted bitter	- no remarkable evidence - needle puncture mark

mate of the deceased had also recreationally taken mephedrone and heroin, but did not die. The fact that one individual had died while the other had survived indicates the complexity in predicting adverse effects after multiple drug taking.

Several fatalities were reported in the media across Europe, initially implicating mephedrone misuse, but few have actually resulted in mephedrone being cited as the cause of death[29,30]. This coverage in the 'lay press' tends to occur when someone dies, and is generally based on anecdotal reports of mephedrone use. In general, at the time of death, when the media's interest is maximal, there are no toxicological findings available to support the claims that mephedrone was linked to the death, since toxicological analysis of ante- or post-mortem biological samples takes time. The first death attributed to lone mephedrone toxicity was in an 18-year-old female in Sweden[15], where toxicological screening of blood and urine revealed the presence of mephedrone with no other drugs or alcohol detected. However, the mephedrone concentration was not reported.

It is worth pointing out the upmost importance of investigators and medical examiners to provide relevant information and the circumstances of their cases to the forensic toxicology laboratory. If a case in which the individual was known to have been using mephedrone or any new designer drugs is submitted without a specific request for the corresponding testing, the outcome could be negative, therefore new drugs may be left unidentified, consequently leading to an incomplete determination of death by the medical examiners. Thus, Torrance and Cooper[35] suggest that "Forensic Toxicology laboratories must assess their current testing protocols to ensure they can meet the demand for both a targeted quantitative analysis and a suitable screening regime that will ensure cases submitted for analysis will detect the presence of the mephedrone in a range of biological matrices".

Conclusions

Data currently available suggest that the abuse of mephedrone is still a public health issue even after its classification[32]. Case reports indicate that there is no safe mephedrone dose, since negative consequences may appear in association with any dosage taken. Moreover, similar dosages may have markedly different side effects in different

subjects. In addition, mephedrone appears to have a rather narrow therapeutic window that makes its use dangerous. Dosages which supposedly fall within recreational use limits could also lead to death when combined with other drugs in certain circumstances. Accidental deaths attributed to mephedrone poisoning, where the blood concentrations were close to those found in the living, support the latest view[39].

Therefore, it is of upmost importance of investigators and medical examiners providing relevant information and the circumstances of their cases to the forensic toxicology laboratory. Finally, Forensic Toxicology laboratories must assess their testing procedures to ensure they can achieve both an appropriate screening regime and targeted quantitative analysis for the detection of mephedrone in various biological matrices.

Conflict of Interest

None of the authors have any conflict of interest, including specific financial interests, relationships or affiliations relevant to the manuscript.

References

1) King LA, Kicman AT. A brief history of 'new psychoactive substances'. Drug Test Anal 2011; 3: 401-403.
2) Johnson LA, Johnson RL, Portier RB. Current "legal highs". J Emerg Med 2013; 44: 1108-1115.
3) Elliott S, Evans J. A 3-year review of new psychoactive substances in casework. Forensic Sci Int 2014; 243: 55-60.
4) Collins M. Some new psychoactive substances: precursor chemicals and synthesis-driven end-products. Drug Test Anal 2011; 3: 404-416.
5) Gibbons S. 'Legal highs'--novel and emerging psychoactive drugs: a chemical overview for the toxicologist. Clin Toxicol (Phila) 2012; 50: 15-24.
6) Prosser JM, Nelson LS. The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 2012; 8: 33-42.
7) Ayres TC, Bond JW. A chemical analysis examining the pharmacology of novel psychoactive substances freely available over the internet and their impact on public (ill)health. Legal highs or illegal highs? BMJ Open 2012; 2: pii e000977.
8) Weaver MF, Hopper JA, Gunderson EW. Designer drugs 2015: assessment and management. Addict Sci Clin Pract 2015; 10: 8.
9) Patel NB. Mechanism of action of cathinone: the active ingredient of khat (Catha edulis). East Afr Med J 2000; 77: 329-332.
10) Kelly JP. Cathinone derivatives: a review of their chemistry, pharmacology and toxicology. Drug Test Anal 2011; 3: 439-453.
11) Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Ruoho AE, Cozzi NV. The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 2012; 37: 1192-1203.
12) 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.
13) Wood D, Davies S, Puchnarewicz M, Button J, Archer R, Ovaska H, Ramsey J, Lee T, Holt DW, Dargan PI. Recreational use of Mephedrone (4-Methylmethcathinone, 4-MMC) with associated sympathomimetic toxicity. J Med Toxicol 2010; 6: 327-330.
14) Regan L, Mitchelson M, Macdonald C. Mephedrone toxicity in a Scottish emergency department. Emerg Med J 2011; 28: 1055-1058.
15) Gustavsson D, Escher C. Mephedrone--Internet drug which seems to have come and stay. Fatal cases in Sweden have drawn attention to previously unknown substance. Lakartidningen 2009; 106: 2769-2771.
16) Loeffler G, Hurst D, Penn A, Yung K. Spice, bath salts, and the U.S. military: the emergence of synthetic cannabinoid receptor agonists and cathinones in the U.S. Armed Forces. Mil Med 2012; 177: 1041-1048.
17) Dickson AJ, Vorce SP, Levine B, Past MR. Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. J Anal Toxicol 2010; 34: 162-168.
18) Poklis JL, Wolf CE, ElJordi OI, Liu K, Zhang S, Poklis A. Analysis of the first- and second-generation Raving Dragon Novelty Bath Salts containing methylone and pentedrone. J Forensic Sci 2015; 60: S234-240.
19) Schifano F, Albanese A, Fergus S, Stair JL, Deluca P, Corazza O, Davey Z, Corkery J, Siemann H, Scherbaum N, Farre' M, Torrens M, Demetrovics Z, Ghodse AH, Psychonaut Web Mapping, ReDNet Research Groups. Mephedrone (4-methylmethcathinone; 'meow meow'): chemical, pharmacological and clinical issues. Psychopharmacology (Berl) 2011; 214: 593-602.
20) Angoa-Perez M, Kane MJ, Francescutti DM, Sykes KE, Shah MM, Mohammed AM, Thomas DM, Kuhn DM. Mephedrone, an abused psychoactive component of 'bath salts' and methamphetamine congener, does not cause neurotoxicity to dopamine nerve endings of the striatum. J Neurochem 2012; 120: 1097-1107.
21) Vardakou I, Pistos C, Spiliopoulou C. Drugs for youth via Internet and the example of mephedrone. Toxicol Lett 2011; 201: 191-195.
22) Archer RP. Fluoromethcathinone, a new substance of abuse. Forensic Sci Int 2009; 185: 10-20.

23) Busardò FP, Kyriakou C, Tittarelli R, Mannocchi G, Pantano F, Santurro A, Zaami S, Baglìo G. Assessment of the stability of mephedrone in antemortem and post-mortem blood specimens. Forensic Sci Int 2015; 256: 28-37.

24) Camilleri A, Johnston MR, Brennan M, Davis S, Caldicott DG. Chemical analysis of four capsules containing the controlled substance analogues 4-methylmethcathinone, 2-fluoromethamphetamine, alpha-phthalimidopropiophenone and N-ethylcathinone. Forensic Sci Int 2010; 197: 59-66.

25) Carhart-Harris RL, King LA, Nutt DJ. A web-based survey on mephedrone. Drug Alcohol Depend 2011; 118: 19-22.

26) Busardo FP, Kyriakou C. GHB in biological specimens: which cut-off levels should be taken into consideration in forensic toxicological investigation? Recent Pat Biotechnol 2015; 8: 206-214.

27) Busardo FP, Jones AW. GHB Pharmacology and toxicology: acute intoxication, concentrations in blood and urine in forensic cases and treatment of the withdrawal syndrome. Curr Neuropharmacol 2015; 13: 47-70.

28) Cosbey SH, Peters KL, Quinn A, Bentley A. Mephedrone (methylmethcathinone) in toxicology casework: a Northern Ireland perspective. J Anal Toxicol 2013; 37: 74-82.

29) Adamowicz P, Tokarczyk B, Stanaszek R, Slopianka M. Fatal mephedrone intoxication--a case report. J Anal Toxicol 2013; 37: 37-42.

30) Dargan PI, Sedefov R, Gallegos A, Wood DM. The pharmacology and toxicology of the synthetic cathinone mephedrone (4-methylmethcathinone). Drug Test Anal 2011; 3: 454-463.

31) Winstock A, Mitcheson L, Ramsey J, Davies S, Puchnarewicz M, Marsden J. Mephedrone: use, subjective effects and health risks. Addiction 2011; 106: 1991-1996.

32) Winstock A, Mitcheson L, Marsden J. Mephedrone: still available and twice the price. Lancet 2010; 376: 1537.

33) Fass JA, Fass AD, Garcia AS. Synthetic cathinones (bath salts): legal status and patterns of abuse. Ann Pharmacother 2012; 46: 436-441.

34) Favretto D, Pascali JP, Tagliaro F. New challenges and innovation in forensic toxicology: Focus on the "New Psychoactive Substances". J Chromatogr A 2013; 1287: 84-95.

35) Torrance H, Cooper G. The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. Forensic Sci Int 2010; 202: e62-63.

36) Maskell PD, De Paoli G, Seneviratne C, Pounder DJ. Mephedrone (4-methylmethcathinone)-related deaths. J Anal Toxicol 2011; 35: 188-191.

37) Lusthof KJ, Oosting R, Maes A, Verschraagen M, Dijkhuizen A, Sprong AGA. A case of extreme agitation and death after the use of mephedrone in The Netherlands. Forensic Sci Int 2011; 206: e93-e95.

38) Rojek S, Klys M, Maciow-Glab M, Kula K, Strona M. Cathinones derivatives-related deaths as exemplified by two fatal cases involving methcathinone with 4-methylmethcathinone and 4-methylethcathinone. Drug Test Anal 2014; 6: 770-777.

39) Wikstrom M, Thelander G, Nystrom I, Kronstrand R. Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). J Anal Toxicol 2010; 34: 594-598.

40) Aromatario M, Bottoni E, Santoni M, Ciallella C. New "lethal highs": a case of a deadly cocktail of GHB and Mephedrone. Forensic Sci Int 2012; 223: e38-41.

41) Wood DM, Davies S, Greene SL, Button J, Holt DW, Ramsey J, Dargan PI. Case series of individuals with analytically confirmed acute mephedrone toxicity. Clin Toxicol (Phila) 2010; 48: 924-927.