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Synthetic cathinones – From natural plant stimulant to new drug of abuse

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ABSTRACT

As recreational substances, synthetic cathinones started to be used at the beginning of the 21st century. There is still limited data on these compounds, introduced to the illicit drug market for the most part after 2009. Considering that synthetic cathinones are currently the second largest group of new psychoactive and dangerous substances among over 670 new psychoactive substances identified in Europe and monitored by the EMCDDA, research on them should be regarded as extremely important.

This review focuses on the availability of synthetic cathinones on the illicit drug market, presentation of current trends in the use of these substances, and their mechanisms of action and toxicity. The authors discuss cases of intoxication with synthetic cathinones and post-mortem diagnostics as well as the problem of combined used of synthetic cathinones with other psychoactive substances.

Literature as well as clinical and forensic data indicate the need for further research on the metabolism, toxicokinetics, toxicodynamics, clinical effects, and addictive potential of synthetic cathinones, especially in the context of potential threats caused by increased consumption of this group of drugs in future.

1. Introduction

Many new psychoactive substances (NPSs) were created throughout the 1960s and the remainder of the 20th century. While they may not have been referred to specifically as NPSs, drug analogues have been continually developed for decades. As recreational substances, NPSs started to be used on a large scale at the beginning of the 21st century. United Nations Office on Drugs and Crime (UNODC) defines NPSs as 'substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat'. This definition, therefore, excludes all internationally classified substances, including 3,4-methylenedioxymethamphetamine (MDMA) and amphetamines. The term 'new' does not necessarily refer to new inventions—several NPSs were first synthesized 40 years ago—but to substances that have recently become available on the market (UNODC). These compounds affect the human central nervous system (CNS) in a manner similar to drugs that have been known for a long time, such as amphetamine, cannabis, heroin, or LSD. NPSs are a heterogeneous group of natural, semi-synthetic, and synthetic compounds. The most popular NPSs include synthetic cathinones and synthetic cannabinoids. Over the past few years, the illicit drug market has seen an increase in the number of psychostimulants known as NPSs psychoactive substances, designer drugs, bath salts, plant food, research chemicals, vacuum freshener, pond cleaner, jewelry cleaner, or insect repellent (Zawilska et al., 2013).

In order to avoid criminal liability, preparations containing synthetic cathinones are usually labelled 'substance not suitable for human consumption', 'not tested for safety or toxicity', 'keep out of reach of children', etc. These preparations are purchased mainly in online stores and less often in brick-and-mortar shops or directly from people who distribute them. The composition of products offered under the same trade names may vary with regard to the amount (concentration) of psychoactive substances affecting the CNS and they may even contain other types of substances than those specified on their labels, which means that their users do not always know what substance they consume and in what quantity. Both quality and quantity of synthetic cathinones available on the drug market are inadequate (Adamowicz

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et al, 2016a,b; Odoardi et al., 2016). Many products increasingly contain not only single active ingredients but also combinations of two or more derivatives. They often include admixtures, e.g. caffeine and lidocaine (Capriola, 2013). Synthetic cathinones are sometimes sold as cocaine or ecstasy (MDMA) (EMCDDA, 2015b).

On the illicit market, synthetic cathinones are usually distributed in the forms of white powder, crystals, or capsules, while the tablet form is less popular. Synthetic cathinones are usually taken orally (capsules, water solutions, and tablets) and nasally (snorting of a powdered substance and its absorption through the nasal mucosa). In order to absorb a single large dose of a synthetic cathinone, users swallow the substance wrapped in cigarette paper (so-called 'bombing') (German et al., 2014; Prosser et al., 2012). Due to high water solubility, synthetic cathinones are also taken by intravenous and intramuscular injections and used in the form of enemas. It has been reported that in some cases intravenous injections were followed by venous thromboses and embolisms as well as local infections, abscesses, scabs, and scars (EMCDDA, 2015a). Opioid injectors also often declare injecting synthetic cathinones (EMCDDA, 2015b; Péterfi et al., 2014). There have been several reports warning of the rising popularity of intravenous use of synthetic cathinones (EMCDDA, 2015a). Synthetic cathinones are sometimes used in so-called 'mephedrone sessions', which means taking substances in several repeated doses within a few hours and usually in a specific social situation (e.g. at friends' homes, at a home party, or in nightclubs) (German et al., 2014).

Users of synthetic cathinones give a number of reasons for taking these substances, including the legal status (legality), availability (purchased mainly via the Internet), acceptable price (lower compared to conventional drugs), lack of rapid screening tests to confirm their intake, or user preferences for specific pharmacological properties, e.g. they are taken to enhance social and sexual experiences (Benschop et al., 2017).

Due to the initial legal status of synthetic cathinones, they were regarded as 'legal highs' and often considered by users as safe alternatives to other commonly abused stimulants. The first drugs from this group on the illicit market were methcathinone and 4-methylmethcathinone (4-MMC, mephedrone), followed by 3,4-methylenedioxymethcathinone (MDMC, methylone), 3,4-methylenedioxypyrovalerone (MDPV), 4-methoxymethcathinone (methedrone), and α-pyrrolidinopropiophenone (PPP) (EMCDDA, 2015b). Initially, mephedrone and MDPV were cathinones most commonly found in 'bath salt' products in Europe. Mephedrone gained its popularity due to, among others, the low quality and difficulties in obtaining cocaine or MDMA (EMCDDA, 2016). The speed with which synthetic cathinones emerged among the wider population of drug users and the scale of their adverse health effects resulted in introduction of legal control over these compounds.

Currently, synthetic cathinones are the second largest group of new psychoactive substances among over 670 NPSs identified in Europe and monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). According to an EMCDDA report, a total of 130 synthetic cathinones were registered in Europe by the end of 2017 and 12 such substances were identified for the first time that year (EMCDDA, 2018). In 2016, synthetic cathinones accounted for almost one-third (over 23,000) of the overall number of seizures of NPSs. The total number of seized synthetic cathinones approaches 1.9 tonnes, putting them in the first place on the list of seized NPSs in 2016 (EMCDDA, 2016).

Reports assessing the demographic data on synthetic cathinone users conclude that the respondents are mostly young males. A review of data collected from six EU countries (Germany, Hungary, Ireland, the Netherlands, Poland, and Portugal) found out that the Internet community interested in the subject of synthetic cathinones consisted of people aged 18 to 25 years; however, the profile of synthetic cathinone users was most likely in the age group of 18–35 years (Benschop et al., 2017).

2. Chemical properties

Synthetic cathinones are structural analogues of cathinone, a psychostimulant alkaloid present in khat (*Catha edulis*). Khat has been known and used for centuries by the people of East Africa and northeastern part of the Arabian Peninsula due to its psychoactive properties. It was discovered in Yemen in the 18th century by the botanist Peter Forskal. According to historical references, the practice of chewing khat leaves for their euphoric and stimulant effects dates back many centuries; today, it is still popular in such countries as Somalia, Yemen, Kenya, and Ethiopia (Ageely, 2008). Khat leaves contain multiple compounds, particularly phenylalkylamine alkaloids that include norpseudoephedrine, cathinone, and cathine. It was not until the 1970s that cathinone, specifically S-(-)-cathinone stereoisomer, was isolated from khat leaves and determined to be its principal psychoactive component (Kalix, 1990). Cathinone decomposes quickly after leaves are harvested, which is why fresh leaves are chewed only in the countries where the plant grows or in neighbouring countries. The oral mucosa plays a major role in the absorption of alkaloids. Khat leaves are also occasionally cooked in the Arabian Peninsula and in some regions of East Africa in the form of tea (Capriola, 2013; Karila et al., 2015). Many years before the discovery of cathinone in khat, the compound was synthesized by medical chemists (Patel, 2019).

2.1. Physicochemical properties of synthetic cathinones

Synthetic cathinones were synthesized for the first time in the 1920s as potential medicinal products. The first synthetic cathinone—methcathinone—was produced in 1928, followed by mephedrone a year later (Prosser et al., 2012).

A characteristic feature of all synthetic cathinones is the presence of the ketone group in the β-position of the side chain (Fig. 1A). The substituents (R^1-R^5) are most often hydrogen atoms or simple aliphatic chains, but also, among others, pyrrolidine rings or halogen groups.

The chemical structure of synthetic cathinones is similar to amphetamine, methamphetamine, and ecstasy, which is why they are commonly called β-ketoamphetamines (Fig. 1B). All synthetic cathinones are based on the basic structure of natural cathinone and are derivatives of phenylalkylamines, structurally resembling the amphetamine molecule with a carbonyl bond in the β-position of the aminoalkyl chain substituted at the aromatic ring (EMCDDA, 2015b).

From a chemical point of view, the cathinone derivatives can be divided into three groups (Vari et al., 2019). Group 1 consists of *N*-alkyl compounds or those with an alkyl or halogen substituent at any position

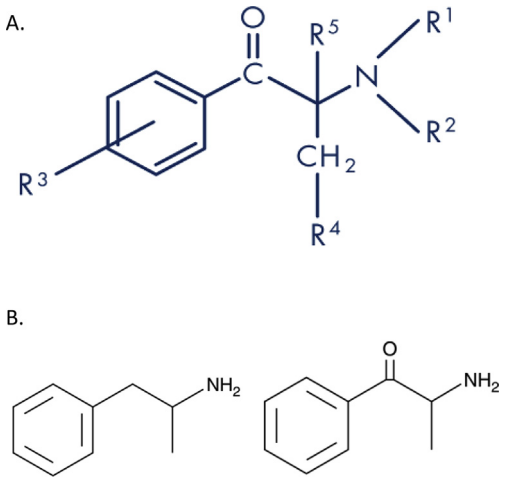

Fig. 1. General structure of a cathinone derivative showing substitution patterns (A) and a comparison of chemical structure (B) of amphetamine (left) and cathinone (right).

of the aromatic ring. It includes the first synthetic cathinones, i.e. buphedrone, ethcathinone, ephedrone, flephedrone, mephedrone, and pentedrone. Group 2 includes methylenedioxy-substituted compounds at any given position of the aromatic ring, such as butylone, methylone, and pentylone. In terms of their structure, these compounds resemble more MDMA and exhibit similar activity. Cathinone derivatives from group 3 are analogues with pyrrolidine derivatives such as 3,4-methylenedioxyalphapyrrolidinopropiophenone (MDPPP) and above mentioned MDPV.

2.2. Analytical methods for identification

NPSs are becoming increasingly popular and tests for their presence in biological material should be part of the routine analysis commissioned by law enforcement authorities. Also, it should be taken into consideration that some cathinones are unstable in biological matrices. The collected biological material may be influenced by various conditions during transport, storage, and analysis of samples, which may cause significant changes in the concentration of xenobiotics before their analysis. Analysis of the stability of synthetic cathinones in blood at temperatures of −20 °C, 4 °C, 20 °C, and 32 °C showed that it depends not only on the storage temperature of the biological material but also on the chemical structure of a given substance, and especially the presence of substituents in the aromatic ring and at the nitrogen atom (Glicksberg and Kerrigan, 2017). At a temperature of 32 °C, a significant analyte loss was observed in just a few hours. Similar studies carried out on urine samples showed dependence of the stability of synthetic cathinones on urine pH and storage temperature (Glicksberg and Kerrigan, 2018; Adamowicz and Malczyk, 2019). Cathinones were much more stable in acidic urine (pH = 4) and in low-temperature conditions. In alkaline urine (pH = 8) and at a temperature of 32 °C, a significant analyte loss (> 20%) was observed in just a few hours.

Currently, among the research methods used to analyse designer drugs, including synthetic cathinones, only advanced chromatographic techniques allow unambiguous identification and quantification of psychoactive substances in biological material collected from living persons or secured during autopsy. Forensic toxicology laboratories most commonly use liquid and gas chromatography coupled with mass spectrometry (LC-MS and GC-MS) (Levitas et al., 2018; Swortwood et al., 2013).

Analytical problems related to the identification of psychoactive substances that are part of 'designer drugs' result mainly from the large and constantly increasing number of these substances in the global and domestic drug markets. Apart from their diversity, NPSs also pose great analytical difficulties due to their action on the body in small doses as well as rapid and numerous metabolic changes that they undergo in the body. This leads to low concentrations of parent compounds and their numerous metabolites in biological material. Therefore, the methodology used in toxicology laboratories must by regularly updated to keep up with the rapidly changing market (Adamowicz et al., 2016a).

One of the main difficulties in toxicological analysis is to distinguish compounds having similar chemical structures, including structural isomers, i.e. chemical compounds with identical molecular formulas, which differ in the type, order, or spatial arrangement of atomic bonds. This is illustrated on the example of 4-chloromethcathinone (clephedrone, 4-CMC) and its isomers 2-CMC and 3-CMC shown in Fig. 2.

These compounds differ only in the location of the methyl group within the benzene ring in their structures, which generates similar chemical properties, but also similar analytical (chromatographic) properties based on which we definitively identify a given substance. Despite their structural similarities, positional isomers may exhibit different pharmacological properties and significantly different toxicity. Separation and correct identification of isomers is also extremely important in relation to the legal status of a given substance, which may vary.

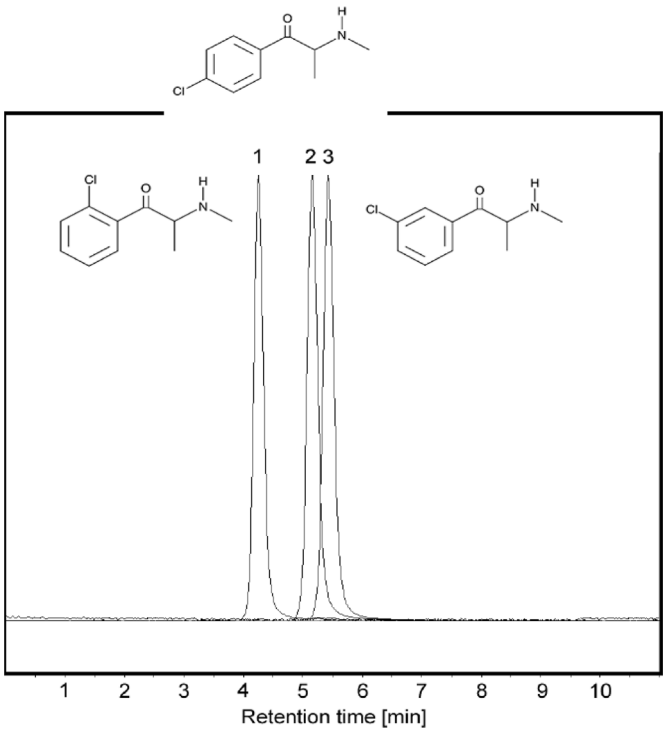

Fig. 2. LC-MS/MS chromatogram of chloromethcathinone isomers: (left (1) to right (3)): 2-chloromethcathinone (2-CMC), 4-chloromethcathinone (4-CMC), and 3-chloromethcathinone (3-CMC) as examples of similar structural formulas of synthetic cathinones. LC-MS/MS technique allows to distinguish constitutional isomers of chloromethcathinone.

3. Pharmacoclinical effects

Each year, up to several dozen psychoactive substances enter the drug market on which there is little or no literature data concerning their physicochemistry, pharmacology, or toxicity. There is a lack of detailed data on the effects of synthetic cathinones on the human body, although they are known to exhibit many structural, pharmacological, and behavioural effects similar to amphetamine and its derivatives (Simmler et al., 2014).

Some cathinones, for example pyrovalerone derivatives, were originally synthesized as new therapeutic agents but were later withdrawn and classified as NPSs (Meltzer et al., 2006). Like amphetamine, cathinone and its synthetic derivatives increase the levels of brain monoamines, such as noradrenaline (NE), serotonin (5-HT), and dopamine (DA). Similar to phenethylamines, such as MDMA, synthetic cathinones may have both amphetamine-like properties and the ability to modulate 5-HT, resulting in pronounced psychoactive effects. Most cathinone derivatives exhibit sympathomimetic effects. Other qualities, including the duration and range of psychoactive effects, vary largely depending on the structure of the functional group. For example, shortening of the alkyl chain reduces dopamine transporter activity (Kolanos, 2015). It has also been shown that synthetic cathinones are more hydrophilic than their amphetamine counterparts and are less able to cross the blood-brain barrier (EMCDDA, 2015b).

The usefulness of synthetic cathinones in medicine has been studied primarily in terms of use of these compounds as antidepressants and anorectic drugs (AMCD, 2010). Diethylpropion, also known as amfepramone, which is an N,N-diethyl analogue of cathinone, was developed as an anorectic agent in the early 1960s by the German pharmaceutical company Temmler-Werke (Regenon, 2009) and is still prescribed in some countries as Tenuate, an effective preparation for weight loss (Suplicy, 2014). Pyrovalerone was investigated as an antifatigue agent in the 1960s (Seeger, 1967). Bupropion is an N-tert-butyl

analogue of cathinone that was initially investigated as an antidepressant in the 1970s by Burroughs Wellcome (now GlaxoSmithKline), and subsequently approved for clinical use as Wellbutrin (Dhillon et al., 2008). Bupropion was also approved as the smoking cessation aid Zyban in 1997 (Dwoskin, 2006). In 2016, bupropion was the fifth most prescribed psychiatric drug in the USA (Grohol, 2016).

Consumers of psychoactive NPSs expect them to have a number of effects, in particular good mood, increased self-confidence, euphoria, ease of interpersonal communication, increased sensual perception and openness in expressing one's feelings, increased energy and psychophysical activity, increased concentration, 'clarity of thought', improved cognitive functions and the ability to remember, and reduced drowsiness.

Recently, a new trend has emerged, consisting of injecting illegal drugs, including synthetic cathinones, in a specific context, a practice that is referred to as 'slamming'. This term describes intravenous injection of psychoactive substances at events where people engage in sex to increase sexual desire and pleasure. Usually, this takes place at the so-called 'chem-sex' parties, where synthetic cathinones are used simultaneously or in combination with other psychoactive substances, such as methamphetamine, GHB/GBL, cocaine, or sildenafil (Viagra) (Pellegrini et al., 2019; Troya et al., 2019). These events can last from several hours to even three days, and the participants often engage in risky sexual behaviour, such as not using condoms and having sex with many casual partners. Such risky behaviour can lead to common medical and psychiatric problems associated with intravenous drug use, including contraction of sexually transmitted diseases (STDs) or viral infections, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) (Dolengevich-Segal et al., 2016). These complications are not related to the pharmacological effects of cathinones but to the routes of administration and lack of sterility.

The above recreational effects of synthetic cathinones have been observed in users, particularly in younger age groups (Riley et al., 2019). However, synthetic cathinones exert a variety of adverse or even toxic effects on the human body (Karch, 2015; Logan et al., 2017; Nóbrega et al., 2018; Riley et al., 2019). Among them, the most commonly reported are cardiovascular symptoms, such as tachycardia, increased blood pressure, palpitations, chest pain, myocarditis, and cardiac arrest; neurological symptoms, such as insomnia, headaches, teeth grinding, seizures, visual disturbances, and paraesthesia; and others, such as nosebleeds, ulcerations of the mucous membranes of the nose and throat, skeletal muscle breakdown (rhabdomyolysis), and kidney damage. Other possible effects include disseminated intravascular coagulation (DIC) and multiple organ failure leading to death. In the case of mephedrone and MDPV, there have been reports of thromboembolic complications and local infections (including necrotizing fasciitis).

Changes in behaviour and perception of the world that occur after taking synthetic cathinones and go beyond those expected by the consumer include a variety of mental and cognitive disorders (Capriola, 2013; Zawilska, 2014). A person under the influence of cathinones may exhibit irritability, aggression (sometimes manifesting in extreme violence), self-harm, anxiety, panic attacks, lack of motivation, anhedonia, depression, suicidal thoughts and attempts, paranoid delusions, auditory and visual hallucinations (often in the form of people who pose a threat, follow, or intend to kill the user), and even catatonia (De-Giorgio et al., 2019; Richman et al., 2018; Zawilska et al., 2013). With respect to the CNS of intoxicated people, the symptoms identified so far include, among others, confusion, agitation, psychosis, muscle cramps, dizziness, tinnitus, short-term memory impairment, parkinsonism, motor automatisms, cerebral oedema, violent behaviour, and addiction (Dervaux et al., 2017; Hunter et al., 2018; Riley et al., 2019; Zawilska and Wojcieszak, 2017).

As psychoactive and recreational substances, cathinones have significant abuse potential, which was confirmed in behavioural studies in animals and humans. A study by Aarde et al. provided evidence of stimulant-typical abuse liability for 4-MMC in the traditional preclinical rat self-administration model (Aarde et al., 2013). In another study, the substituted cathinone stimulants mephedrone and methylone as well as MDMA were intravenously self-administered by female Wistar rats. Based on the obtained results, it was predicted that the liability of these three compounds is similar in established stimulant users but may differ in liability if they are primary drugs of initiation (Creehan et al., 2015). Also, case reports were published on patients meeting the current DSM-5 criteria for substance use disorders (Johnson et al., 2013; Lev-Ran, 2012; Prosser et al., 2012). Cathinones can induce a significant craving similar to classic drugs of abuse and other withdrawal symptoms, such as sleeping difficulties, anxiety, depression, suspiciousness, tremors, and even psychotic-like behaviour (delusions and/or hallucinations) (Brunt et al., 2011; Prosser et al., 2012).

Synthetic cathinones can also induce psychotic symptoms similar to positive symptoms in schizophrenia (John et al., 2017; Stiles et al., 2016; Thornton et al., 2012). According to John et al., abuse of synthetic cathinones (bath salts) should be in the differential diagnosis where psychosis is of new onset or clinically incongruent with known primary presentation of a psychotic disorder. People under the influence of synthetic cathinones may also have suicidal thoughts and tendencies (Thornton et al., 2012).

Sets of clinical symptoms indicative of intoxication with synthetic cathinones (so-called toxidromes) consist primarily of sympathomimetic and serotonin toxidromes (Table 1). Sympathomimetic toxidrome is a heterogeneous set of clinical symptoms that result from a very strong stimulation of the sympathetic nervous system as a result of taking a sympathomimetic xenobiotic. Serotonin syndrome, in turn, includes various non-specific clinical symptoms resulting from stimulation of serotonergic transmission in the CNS and in peripheral tissues. Symptoms associated with sympathomimetic and serotonin toxidromes are similar. Diagnosis of serotonin syndrome using the Hunter Serotonin Toxicity Criteria involves the administration of a serotonergic agent (e.g. synthetic cathinone) and occurrence of one or more of the following symptoms: (i) spontaneous clonus, (ii) inducible or ocular clonus with agitation and diaphoresis, (iii) tremor and hyperreflexia, or (iv) hypertonia, hyperpyrexia (temperature 38 °C), and inducible or ocular clonus (Dignam et al., 2017).

During drug sessions, synthetic cathinones are often taken with popular medications and other drugs, which multi-directionally modifies their action at the somatic and mental levels, including with alcohol and beta blockers (to prevent tachycardia), cannabis or benzodiazepine

Table 1
The most common toxidromes and their symptoms occurring in the course of intoxication with synthetic cathinones (acc. to Dignam et al., 2017; modified).

Type of toxidrome	Sympathomimetic	Serotonin
Nervous system	Psychomotor agitation	
	Tremors, convulsions, hyperreflexia	Confused state, from drowsiness or somnolence to coma, increased muscle tension
Pupils	Dilated	
Cardiovascular system	Tachycardia, increased blood pressure	
Respiratory system	Tachypnoea	
Temperature	Elevated	
Others	Anorexia, diaphoresis	Diaphoresis, diarrhoea, lockjaw

Table 2
Evaluation of the toxicity of different doses of cathinones in *in vivo* animal studies and *in vitro* cell line studies.

Type of the study	Cathinone	Description of the study	Results	Ref.
In vivo	khat extract	three groups of mice were treated with aqueous solution of khat extract in the doses of 50, 100, and 200 mg/kg	the results indicated a dose-dependent decrease in body weight, an increase in the incidence of mortality, and induction of site-specific body and eye lesions	al-Meshal et al. (1991)
In vitro	methylone and MDPV	undifferentiated and differentiated SH-SY5Y cells were exposed for 24 h to MDMA, methylone, or MDPV at a wide concentration range, from 0.01 to 12 or 20 mM	methylone and MDPV induced loss of cell viability in a concentration-dependent manner, in the following order of potency: MDPV ≈ MDMA > methylone	Valente et al. (2017)
In vitro	cathinone phthalimide (CP)	pheochromocytoma cells were exposed to CP (10 μM – 1,000 μM)	exposure to CP induced cell death and altered mitochondrial function as well as intracellular DA and 5-HT levels; at the same time, reduced glutathione levels remained unaffected	Lantz et al. (2017)

derivatives (to combat anxiety), famotidine, omeprazole, or domperidone (to inhibit gastric acid secretion and prevent abdominal pain), other psychostimulatory compounds, e.g. cocaine, amphetamine, modafinil, trifluoromethylphenylpiperazine, or benzylpiperazine (to increase CNS stimulation and empathy), and ketamine or zopiclone (to intensify visual hallucinations) (Zawilska, 2014).

In vivo experimental studies on animals confirm that synthetic cathinones, presumably by acting on the central monoamine systems, cause profound behavioural changes in animals. Den Hollander et al. investigated the possible long-term effects of mephedrone and methylone on the state of memory, anxiety, and depression in mice as well as the long-term effects of these substances on brain neurochemistry in both rats and mice (Den Hollander et al., 2013). Mephedrone and methylone were administered twice per day for 4 day at doses of 30 mg/kg. After two weeks, behavioural tests were conducted on the effects of these substances on memory, anxiety, and depression; additionally, measurements were taken of the levels of DA, 5-HT, and their metabolites as well as NE in the animal brains. It was demonstrated that mephedrone reduced working memory performance but did not affect neurotransmitter levels except for a 22 per cent lowering of homovanillic acid (HVA) concentration in mice. Methylone had little effect on the behaviour and neurotransmitter levels in mice but caused widespread depletion of 5-HT and 5-HTTT levels in rats. Both methylone and mephedrone were found to have long-term effects on behavioural and biochemical indicators of rodent neurotoxicity.

It was also shown that mephedrone, methylone, and MDPV rapidly increase locomotor activity in a manner described as recurrent bouts of hyperlocomotion separated by brief periods of rest (Baumann et al., 2012; Marusich et al., 2012; Wright et al., 2012a). Differences in mephedrone and MDPV locomotor activities were observed depending on the applied dose of a synthetic cathinone. Mephedrone dose-dependently decreased the intensity and duration of voluntary wheel running, whereas low doses of MDPV increased the intensity and duration of this activity (Huang et al., 2012).

Despite the loss of motor skills, animals receiving mephedrone demonstrated a slight improvement in the growth of visuospatial memory (Wright et al., 2012b). However, short-term improvement in visuospatial memory may not persist for long periods or during chronic exposure to mephedrone. Multi-day administration of mephedrone led to impairment of working memory in mice (Den Hollander et al., 2013). Juvenile rats receiving mephedrone over prolonged periods also showed deterioration of long-term working memory (Motbey et al., 2012). Many of these observations were made in people using mephedrone, but long-term effects of mephedrone use on human memory and behaviour are unknown.

Also, recent animal studies using MDPV have confirmed that taking this substance may cause deficits in object recognition and working memory (Bernstain et al., 2019; Sewalia et al., 2018). The study by Sewalia et al. used a Sprague-Dawley rat model of long-term voluntary binge-like self-administration of MDPV in five 96-h sessions (Sewalia et al., 2018). Compared to animals self-administering saline, animals self-administering MDPV demonstrated (1) robust drug intake that escalated over time, (2) deficits in novel object recognition but not in spatial object recognition, and (3) neurodegeneration in the perirhinal and entorhinal cortices. Bernstain et al. have proved that chronic exposure of animals to MDPV produces site-specific dysregulation of dopamine markers in the mesocorticolimbic circuit and memory deficits in the novel object recognition test that are influenced by D1 receptors (Bernstain et al., 2019).

4. Toxicity

Toxicity of synthetic cathinones is directly related to the structure (chemical composition) of a given substance (Glennon and Dukat, 2016) and also depends on the taken dose of a single substance or mixture. Mechanisms of action of synthetic cathinones are studied in

Table 3
The inhibitory effect of selected cathinone derivatives and classic drugs on DAT, NET, and SERT monoamine transporters. IC50 means substance concentration at which the transporter is 50 per cent blocked. The lower the IC50 value, the greater the substance affinity for the transporter. The DAT/SERT ratio = (1/DAT IC50)/(1/SERT IC50). A low DAT/SERT ratio (< 0.1) indicates a relatively larger serotonergic rather than dopaminergic component of a given agent, similar to MDMA. A high DAT/SERT ratio (> 10) means a relatively greater dopaminergic rather than serotonergic effect of a given agent, similar to methamphetamine. Classic drugs are marked in bold. The substances are ordered according to the increasing DAT/SERT ratio (acc. to Liechti, 2015 and Simmler et al., 2013; modified).

Substance	Chemical class	Pharmacological class	NET IC50 [μM]	DAT IC50 [μM]	SERT IC50 [μM]	DAT/SERT ratio
MDMA	**Amphetamine derivative**	**Empathogen**	**0.45**	**17**	**1.4**	**0.08**
Methedrone	Cathinone derivative	Empathogen	2.2	35	4.7	0.14
Mephedrone	Cathinone derivative	Empathogen–stimulant	0.25	3.3	4.6	1.4
Naphyrone	Cathinone derivative	Empathogen–stimulant	0.25	0.47	0.96	2.0
Cocaine	**Cathinone derivative**	**Stimulant**	**0.45**	**0.8**	**2.4**	**3.1**
Methylone	Cathinone derivative	Empathogen–stimulant	0.54	4.8	16	3.3
Pentylone	Cathinone derivative	Stimulant	0.99	1.3	8.4	6.2
Methamphetamine	**Amphetamine derivative**	**Stimulant**	**0.06**	**1.1**	**24**	**22**
Buphedrone	Cathinone derivative	Stimulant	0.65	4.2	104	25
Methcathinone	Cathinone derivative	Stimulant	0.09	1.1	33	30
Amphetamine	**Amphetamine**	**Stimulant**	**0.09**	**1.3**	**52**	**40**
MDPV	Pyrovalerone–cathinone	Stimulant	0.04	0.03	9.3	300
α-PVP	Pyrovalerone derivative	Stimulant	0.02	0.05	301	6,020
Methylphenidate	**Pipradol derivative**	**Stimulant**	**0.13**	**0.12**	**807**	**6,725**

experimental animal models and in *in vitro* cell cultures. However, only a limited number of these compounds have been tested (Table 2). Analysis of the results of these studies is complex not only because of differences in conducted experiments but also because of use in individual studies of various substances from the group of synthetic cathinones, typically those most popular on the illicit drug market. A number of descriptions in the literature of toxic effects caused by synthetic cathinones relate to the so-called first generation, which includes 4-MMC, MDMC, and MDPV (Karch, 2015). To date, no *in vitro* tests have been performed on some synthetic cathinones, even on substances identified on the illicit drug market before 2010, including ethcathinone, dimethylcathinone (metamfepramone), and benzedrone. There are not many data even for the newer generation of synthetic cathinones, such as N-propylcathinone, 4-fluoroethcathinone, and 3′,4′-trimethylene-α-ethylaminovalerophenone (Wolff, 2017).

4.1. Mechanism of action of synthetic cathinones with particular emphasis on the CNS

The molecular mechanisms of action of synthetic cathinones mimic the action of classic drugs and rely primarily on increasing extracellular concentration of monoaminergic neurotransmitters: DA, NE, and 5-HT (Banjaw et al., 2006; Baumann et al., 2018; Eshleman et al., 2019; Iversen et al., 2013).

Generally, considering the mechanism of action and similarity to classic drugs, synthetic cathinones are divided into three subgroups.

- The first subgroup consists of cathinones with similar effects to cocaine and ecstasy (e.g. mephedrone, methylone, ethylone, and butylone). Like cocaine, these compounds inhibit reuptake of monoamines, in particular DA. In addition, they imitate the action of ecstasy by stimulating the release of 5-HT.
- The second subgroup consists of methamphetamine-like cathinones (e.g. methylcathinone and flephedrone), which, like amphetamine and methamphetamine, inhibit the reuptake of DA and NA and stimulate release of DA.
- The third subgroup are pyrovalerone cathinones (e.g. MDPV, a-PVP, PV8), which strongly inhibit DA and NA reuptake, but they do not affect the release of monoamines (Calinski et al., 2019; De Felice et al., 2014; Zawilska and Wojcieszak, 2013).

The regulation of concentrations of monoaminergic neurotransmitters takes place mainly at the level of membrane transport proteins responsible for reverse transport of neurotransmitters from the synaptic cleft into the neuron (respectively, dopamine transporter – DAT, noradrenaline transporter – NET, and serotonin transporter – SERT). Compounds that are inhibitors of transport proteins temporarily inhibit reuptake of monoamines from the synaptic cleft into the neuron and thus increase their concentration in the synaptic cleft. *In vitro* studies showed that mephedrone and methylone inhibit DAT, SERT, and NET in micromolar concentrations, and α-PVP and MDPV are potent inhibitors of DAT and NET in the nanomolar range. Effects on other neurotransmitter receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid – AMPA, cannabinoid – CB, γ-aminobutyric acid – $GABA_A$, N-methyl-D-aspartate – NMDA, nicotinic acetylcholine receptor – nACh-R) have not been reported (Hondebrink et al., 2018).

Synthetic cathinones produce various effects on markers of monoaminergic terminal function; they can increase the formation of reactive oxygen and nitrogen species, induce apoptotic signalling, and cause neurodegeneration and cytotoxicity. There is some evidence that mephedrone, MDPV, and methylone alter SERT and DAT levels and, thus, exhibit the potential to damage these nerve terminals; however, additional studies on synthetic cathinone-induced neuroinflammation and neurotoxicity are clearly needed (Leyrer-Jackson et al., 2019).

Synthetic cathinones generally show poor affinity to trace amine-associated receptor 1 (TAAR1), which is targeted by other amphetamines. The exceptions are 2,4-dimethylmethcathinone and 2,3-dimethylmethcathinone, which show submicromolar affinity to TAAR1 receptors in mice and rats (Luethi et al., 2017).

Psychoactive substances showing psychostimulatory and euphoric effects intensify dopaminergic and noradrenergic transmission, whereas empathogenic substances, such as MDMA or 3,4-methylenedioxy-N-ethylamphetamine (MDEA), intensify serotonergic transmission. The parameter that allows to predict the nature of a psychoactive substance is the ratio between its affinity to DAT and its affinity to SERT (so called DAT/SERT ratio) (Liechti, 2015). The lower the value, the greater the

share of the serotonergic component (Table 3). Some cathinones also act as substrates of transport proteins and, when transferred to the inside of neurons, additionally release neurotransmitters into the synaptic cleft (Smith et al., 2015).

In the latest European drug report, some new synthetic cathinones emerged, such as 4-CMC and 4-chloroethcathinone (4-CEC) (EMCDDA, 2019). 4-CMC is a para-halogenated methcathinone derivative. Luethi et al. published a study presenting the pharmacological profile and hepatocellular toxicity of para-halogenated amphetamines and cathinones. Monoamine uptake inhibition was evaluated in transfected human embryonic kidney cells (HEK 293) that expressed the human DAT, SERT, and NET. It was shown that 4-CMC and flephedrone (4-fluoromethcathinone 4-FMC) inhibited norepinephrine uptake and dopamine uptake in micromolar concentrations. The following toxicity rank order for the para-substituents was observed: chloride > fluoride > hydrogen (Luethi et al., 2019).

Similar results were observed with other halogenated cathinones and pyrovalerone cathinones. Methcathinone, 4-fluoromethcathinone, 4-bromomethcathinone, 4-ethylmethcathinone, 4-methylmethcathinone, pyrovalerone, 3,4-methylenedioxy-alpha-pyrrolidinopropiophenone (MDPPP), 3,4-methylenedioxy-alpha-pyrrolidinobutiophenone (MDPBP), MDPV, naphyrone, and α-PVP were tested for the released monoamines: 5-HT, DA, and NE, and for monoamine reuptake transporter inhibition as markers of toxicity. Toxicity was assessed in 293 (HEK 293) cells. 4-methylmethcathinone, 4-ethylmethcathinone, 4-bromomethcathinone, and 4-fluoromethcathinone released 5-HT, DA, and NE. Methcathinone released only DA and NE (Rickli et al., 2015). It suggested that para-halogenation or para-addition of methyl and ethyl groups to methcathinone act on 5-HT release. Pyrovalerone and pyrovalerone-type cathinones (MDPPP, MDPBP, MDPV, naphyrone, α-PVP) did not release DA, NE, or 5-HT. 4-bromomethcathinone and 4-ethylmethcathinone were very potent NET, DAT, and SERT inhibitors. Very potent inhibitors of NET at the lowest concentrations were methcathinone, 4-fluoromethcathinone, 4-bromomethcathinone, and 4-methylmethcathinone. Methcathinone, 4-methylmethcathinone, and 4-fluoromethcathinone were more potent DAT inhibitors than 4-bromomethcathinone and 4-ethylmethcathinone. Methcathinone, 4-methylmethcathinone, and 4-fluoromethcathinone were poorer inhibitors of 5-HT than 4-bromomethcathinone and 4-ethylmethcathinone. All of the pyrovalerone cathinones were very potent catecholamine transporter (NET and DAT) inhibitors with very low serotonergic activity. It should be considered that if any synthetic cathinones are involved in inhibition of monoamine release, they could be potent inhibitors of the corresponding monoamine transporter. However, more detailed studies are needed to prove this hypothesis.

It should also be noted that a number of studies have found active metabolites of MDPV and mephedrone to be important modulators of catecholamine transporter action. Tests of biological samples showed that MDPV is metabolised to 3,4-dihydroxypyrovalerone (3,4-catechol-PV) and 4-hydroxy-3-methoxypyrovalerone (4-OH-3-MeO-PV) (Baumann et al., 2017). The main metabolite of MDPV is 4-OH-3-MeO-PV, which turned out to be a weak blocker in uptake inhibition assays for DAT and NET. On the other hand, 3,4-catechol-PV is a potent uptake blocker at DAT *in vitro* but has little activity after administration *in vivo*. These findings show that MDPV and its active metabolites represent a unique class of transporter inhibitors with high addictive potential (Baumann et al., 2017).

On the other hand, mephedrone acting as a substrate-type releaser at DAT, NET and SERT is metabolised to several phase I compounds, including 4-methylcathinone (nor-mephedrone), 4-hydroxytolylmephedrone (4-OH-mephedrone), and dihydromephedrone. It has been shown that these metabolites of mephedrone are transporter substrates (i.e. releasers) at DAT, NET and SERT, although dihydromephedrone is weak in this regard. When administered *in vivo*, normephedrone increases extracellular dopamine and 5-HT in the brain whereas 4-OH-mephedrone does not. This suggests that 4-OH-mephedrone does not penetrate the blood–brain barrier (Mayer et al., 2016).

Wojcieszak et al. conducted a study on the cytotoxicity of synthetic cathinones from the group of α-pyrrolidinophenone derivatives, including α-PVP, PV8, and PV9 as well as their 4-fluoro- and 4-methoxy derivatives (Wojcieszak et al., 2018). The study was conducted on models of cell lines for the nervous system (SH-SY5Y), liver (Hep G2), and upper airway epithelium (RPMI 2650) as well as cardiomyocytes (H9C2(2-1)). The researchers examined the effect of pyrovalerones on plasma membrane fluidity as a potential mechanism of their cytotoxicity. The study demonstrated that α-pyrrolidinophenones with longer side chains and their fluoro- and methoxy-derivatives produce greater maximum cytotoxicity with respect to the mitochondrial activity and cell membrane integrity than the five-carbon α-PVP and its substituted derivatives. It was also proven that changes of fluidity of the interior part of plasma membrane contribute to the cytotoxicity of pyrovalerone derivatives.

4.2. Toxic effects on the cardiovascular system

It is now known that in addition to the CNS, the organs particularly vulnerable to the toxic effects of synthetic cathinones are the heart and kidneys.

With respect to the cardiovascular system of intoxicated people, the symptoms identified so far include, among others, tachycardia, hypertension, chest pain, heart arrhythmia, myocardial infarction, and myocarditis (Dervaux et al., 2017; Hunter et al., 2018; Riley et al., 2019; Zawilska et al., 2017). Toxic effects of synthetic cathinones on the heart muscle may, in extreme cases, result in sudden cardiac death due to functional changes as well as organic changes, e.g. originating from early myocardial ischaemia or focal inflammatory response (Dawson et al., 2012). The symptoms reported by the intoxicated persons and observed by medical staff are non-specific: dyspnoea, chest pain, tachy- and bradycardia, hyper- and hypotension, etc.

The underlying cause of 'electric' deaths as a result of fatal arrhythmias is the fact that synthetic cathinones may prolong the QT interval in the ECG, promoting *torsade de pointes* polymorphic ventricular tachycardia, which, in turn, may cause syncope and sudden cardiac death even in healthy, young people. These symptoms occur most often during physical exertion and high stress. In addition, the ECGs of people intoxicated with NPSs showed, among others, tachycardia, ST-segment changes, atrial fibrillation, and even asystole (Mladěnka et al., 2018).

For these reasons, during the examination and autopsy, and also during additional tests of people suspected of intoxication with NPSs, special attention should be paid to the presence of pathologies in the myocardium. Obviously, further research is also required on the mechanism of cardiotoxicity of these agents as well as reporting of each analytically confirmed case of cardiac death in the course of intoxication with synthetic cathinones.

4.3. Toxic effects on kidneys

Synthetic cathinones may also cause nausea, vomiting, abdominal pain, intestinal motility disorders, hyponatraemia, hypokalaemia, acidosis, rhabdomyolysis, and acute kidney damage (Dervaux et al., 2017; Hunter et al., 2018; Riley et al., 2019; Zawilska et al., 2017).

The kidneys, as organs involved in the metabolism and excretion of xenobiotics, are particularly vulnerable to the primary and secondary toxic effects of NPSs (Mansoor et al., 2017; Nanavati et al., 2017; Pendergraft et al., 2014). Acute kidney injury (AKI) is most often secondary to non-traumatic rhabdomyolysis, which occurs due to seizures, excessive muscle activity (also in the course of psychomotor agitation), toxic effect of a substance on skeletal myocytes, or a combination of several of the above causes. It is usually accompanied by dehydration and hyperpyrexia (body temperature above 41.1 °C) (Luciano and

Perazella, 2014). Primary AKI in the ischaemic form after use of synthetic cathinones is usually caused by acute tubular necrosis due to vasoconstrictive action of cathinones. The direct nephrotoxic effect of this group of synthetic substances also cannot be rule out (Adebamiro and Perazella, 2012).

Clinicians emphasize that differential diagnoses of unexplained acute kidney injuries, especially in young people, should consider adverse effects of NPSs, even in the cases of negative results of toxicological tests (Mansoor et al., 2017). From the medico-legal perspective, it is important to conduct a pre-mortem analysis of the results of laboratory tests for renal function if the intoxicated person was hospitalized and perform a thorough histopathological assessment distinguishing between non-fatal changes and those that may be a post-humous 'artefact' (Bellomo et al., 2012).

5. Cathinone intoxications

5.1. Non-fatal intoxications and interpretation of concentrations

The most important aspect of forensic toxicological detection of acute intoxication cases are cathinone levels determined in serum. For example, Beck at al. presented 114 cases of intoxication with synthetic cathinones, confirmed analytically in the urine or serum of the patients. Apart from MDPV and α-PVP, which were the most popular, eleven other pyrovalerone derivatives were found (Beck et al., 2018). The following were determined most often: 4′-fluoro-α-pyrrolidinopentiophenone (4F-alpha-PVP, range 23–43 ng/ml), alpha-pyrrolidinohexiophenone (alpha-PHP, range: 4–10 ng/ml), alpha-pyrrolidinobutiophenone (alpha-PBP, range: 2–436 ng/ml), 3′,4′-methylenedioxy-alpha-pyrrolidinohexiophenone (MDPHP, range: 3–136 ng/ml), alpha-pyrrolidinopentiothiophenone (alpha-PVT, range: 8–1,060 ng/ml), and 4′-fluoro-alpha-pyrrolidinohexanophenone (4F-alpha-PHP, range: 3–28 ng/ml). All these substances were also determined in the urine, but the result of determination in this biological material is not a marker of acute intoxication.

Analysing the above concentrations of cathinones in serum it can be stated that the range of synthetic cathinones is very diverse and it is difficult to determine the toxic and lethal concentrations. However, it should be remembered that some of these cases would be fatal if not for the rapid implementation of intensive medical care in hospital setting. Some cases concerned chronically addicted people, who thus demonstrated drug tolerance. In addition, some cases also included other psychoactive substances, which complicates interpretation (Beck et al., 2018). Only in 8 patients a single substance was detected in serum and urine: alpha-PBP (161 ng/ml in serum, 305 ng/ml in urine), MDPHP (< 1–14.3 ng/ml in serum, 19–305 ng/ml in urine), 4F-alpha-PVP (43 ng/ml in serum), alpha-PVT (425 ng/ml in serum), and alpha-PHP (1.9 ng/ml in serum, 3 ng/ml in urine). The determined concentrations in cases of simple intoxications with a single pyrovalerone derivative, which did not result in death, were significantly lower than those reported in mixed intoxications with several substances. In these cases, clinical observations showed psychomotor agitation, muscular pain, hypothermia, hypertension, hallucinations, mydriasis, tachycardia, aggressive behaviour, speech disorders, and paraesthesias. These symptoms were dependent on the time of taking of a given substance. The above observations of the symptoms of intoxication are consistent with the findings of other studies on the impact of NPSs on individual systems of the human organism (Capriola, 2013).

In recent years, synthetic cathinones, alone or in mixtures, have been increasingly detected in drivers of different countries. Since the use of synthetic cathinones can cause various types of mental disorders, e.g. paranoia and hallucination, their intake by drivers may lead to serious traffic accidents resulting in death of the participants. In 28 Polish drivers, where α-PVP was the only detected psychoactive substance, the blood concentration range was 6.4–71 ng/ml. No symptom was found that would occur in all drivers. Symptoms such as staggering gait, confusion, talkativeness, surprise, confusion, slurred speech, slow pupillary light reflex, facial skin redness, and wide or narrow pupils occurred when the blood concentration of α-PVP was in the range of 8.2–68 ng/ml (Adamowicz et al., 2016b). Zawilska et al. reviewed 24 cases of driving under the influence of various pyrovalerone derivatives (Zawilska et al., 2017). α-PVP was detected in 8 cases and its concentrations were in the range of 32–230 ng/ml. MDPV alone was present only in one case and its blood concentration was 60 ng/ml. Other pyrovalerone derivatives were present in combination with other substances. In one case, methylone was detected in blood at a concentration of 6.1 ng/ml and α-PVP at a concentration of 63 ng/ml. In other studies, MDPV was found in blood at a concentration of 6 ng/ml. In combination with other substances, the MDPV concentrations ranged from < 10 to 368 ng/ml. Two cases were described, which concerned motorcyclists involved in road accidents. In the first case, in addition to THC and THC-COOH, the study found MDPV and methylone in blood at concentrations of, respectively, 56 ng/ml and 729 ng/ml. In the second case, only MDPV was found in blood at a concentration of 31 ng/ml (Marinetti and Antonides, 2013). All of these studies agree that in cases of driving motor vehicles under the influence of new psychoactive substances from the group of cathinones, the most frequently detected NPSs are pyrovalerone derivatives.

5.2. Fatal intoxications and post-mortem diagnosis

The exact number of fatal intoxications with NPSs remains unknown for a number of reasons. Firstly, not in every case of death due to an unknown cause an autopsy is ordered with collection of appropriate biological material for specialized toxicological studies (Dinis-Oliveira et al., 2010). Secondly, even if such material is secured, the prosecutor's office does not always order appropriate tests. Thirdly, not every toxicological laboratory that receives such biological material is able to determine the NPSs currently on the market. Fourthly, if an intoxicated person was hospitalized, the biological material collected during the autopsy may no longer contain the substance that was the underlying cause of the sudden deterioration of health.

Investigation of the cause and mechanism of death in the event of a suspected fatal intoxication with synthetic cathinones does not differ from the classic post-mortem diagnosis in forensic medicine (Potocka-Banaś et al., 2017). Similarly to clinical medicine, it includes anamnesis (history taking), physical examination, and additional testing. For obvious reasons, a forensic doctor obtains information only from their client, who is usually a prosecutor, the case files made available by the client, and—what is important if the person was hospitalized—from protected medical records. Ideally, the doctor should get familiar with the medical documentation prior to the examination and autopsy, which are the equivalent of a clinical physical examination. This allows to adjust the scope of the autopsy to the particular case and collect optimal material for additional tests.

In practice, material for complementary histopathological and toxicological tests is reserved most often (including alternative material for routinely collected blood, urine, and fragments of kidney and liver, e.g. samples of adipose tissue, brain, lungs, and hair). It is also possible to carry out post-mortem biochemical, microbiological, and genetic tests, if required (e.g. in cases of suspected sepsis) (Rorat et al., 2014, 2015).

Unfortunately, there is no characteristic sectional image in people intoxicated with designer drugs (Kronstrand et al., 2018; Kubo et al., 2017). Most often, the observations are limited to exponents of sudden death with features of acute circulatory failure in the form of brain and lung oedema, internal organ congestion, and blood fluidity. The scope of the encountered lesions depends mainly on the age of the deceased and whether the intoxicated person was hospitalized. In cases of long-term hospitalization in intensive care unit settings, infectious complications are often observed (e.g. pneumonia in the course of respiratory therapy, features of generalized infection).

Microscopic analysis of sections of internal organs collected during

examination and autopsy of victims of designer drug intoxication has only an ancillary role because even at the microscopic level there is no characteristic morphological picture enabling diagnosis of intoxication (Ezaki et al., 2016).

In fatal intoxications, attempts are made to determine whether the synthetic cathinone was taken alone or as a mixture with other substances. Mephedrone, methedrone, and butylone are often taken with other drugs. For example, post-mortem examinations revealed the presence of mephedrone in combination with ethyl alcohol, 3-trifluoromethylphenylpiperazine, MDMA, heroine, and cocaine. In two cases, the presence of methedrone and butylone was detected along with other xenobiotics (Prosser and Nelson, 2012). In all these cases, death was accidental and detection of several psychoactive substances made it difficult to determine the role of synthetic cathinones as the cause of death.

The most extensive study on deaths associated with the use of synthetic cathinones was published by Kraemer et al., who tried to find all cases with determination of post-mortem concentrations of these substances (Kraemer et al., 2019). For example, MDPV was present in 30 cases, including four cases where it was the only xenobiotic, and MDMC was detected in 16 cases, including six without accompanying psychoactive substances. The causes of death were varied, but in almost all cases synthetic cathinones were determined together with other conventional drugs, new synthetic substances, or medications. As a result, interpretation of the role of synthetic cathinones in the pathomechanism of death is a complex process. In each case, examination is required primarily of accurate data on postmortem redistribution, pharmacokinetics, pharmacodynamics, validated methods for determining synthetic cathinones, and interactions with other xenobiotics. These types of data are unfortunately still very rare (Majchrzak et al., 2018).

6. Combined effects of synthetic cathinones and other psychoactive substances

One of the main problems of modern forensic toxicology is invariably the abuse of several psychoactive substances at the same time. The exact reasons why users combine several types of psychoactive substances remain unknown. Unfortunately, people abuse synthetic cathinones along with synthetic cannabinoids or cannabis and animal studies on the effects of these combinations are still lacking.

Some studies allow to compare the pharmacological mechanisms of action between NPSs and amphetamines, including methamphetamine and MDMA. For example, it has been shown that MDPV with methamphetamine has an opposite effect. That means MDPV is non-toxic to DA terminals. Methylone has no damaging effect on the dopamine nerve terminal. However, as in the case of mephedrone, enhanced neurotoxic effects of methamphetamine are evidenced by a greater reduction in TH, DA and DAT levels (Riley et al., 2019). This is in correlation with the widespread phenomenon of polydrug use of a synthetic cathinone along with other synthetic cathinones or conventional drugs (Kraemer et al., 2019), where the effect on human or animal is enhanced or impaired (Lopez-Rodriguez and Viveros, 2019).

Lopez-Rodriguez and Viveros summarized interactions of synthetic cathinones with ethanol, cannabis, synthetic cannabinoids, cocaine, and nicotine (Lopez-Rodriguez and Viveros, 2019) because both nicotine and ethanol are often used by young people at parties and are, therefore, likely to be combined with NPSs such as synthetic cathinones, potentially contributing to the toxic state of the organism. Ethanol and nicotine together with synthetic cathinones were relatively frequently present in postmortem cases (Kraemer et al., 2019). The effects of the combination of ethanol with mephedrone on 5-HT and DA release were tested in two brain regions: the nucleus accumbens and medial prefrontal cortex, where mephedrone plus ethanol potentiated DA and 5-HT release compared to mephedrone alone (López-Arnau et al., 2018). In experimental studies, mephedrone did not compensate for the effects of ethanol-induced spatial memory disorders (De Sousa Fernandes Perna et al., 2016). Moreover, MDPV with ethanol had different effects on the locomotor activity preference in animals compared to mephedrone alone. Mephedrone plus ethanol significantly increased this parameter but MDPV had an opposite effect. It was probably due to decreasing of blood and brain MDPV concentrations in the first 20 min after injection of MDPV and ethanol (Lopez-Rodriguez and Viveros, 2019). Nicotine and mephedrone administered alone were strongly prooxidative, but together they decreased oxidative status (Budzynska et al., 2015).

Studies on the combined use of synthetic cathinones and cannabis or synthetic cannabinoids are rare. Demographic studies have shown that synthetic cathinones are in fact often taken together with marijuana derivatives (Lopez-Rodriguez et al., 2019). Klavž et al. described intoxication with survival of a person who took a total of two synthetic cannabinoids AB-CHMINACA and AB-FUBINACA and three synthetic cathinones alpha-PHP, alpha-PVP, and 4-CMC. Unfortunately, the concentrations of these xenobiotics were not determined (Klavž et al., 2016). Other studies that analytically confirmed the combined consumption of synthetic cathinones and cannabis or synthetic cannabinoids concerned fatal intoxications. Fatal intoxications with combinations of synthetic cathinones and cannabis or synthetic cannabinoids were collected by Kraemer et al. For example, cases were reported where examination revealed methylone and THC or THC-COOH, alpha-pyrrolidinooctanophenone (PV-9) and THC-COOH, or alpha PVP and THC as well as 11-OH-THC and THC-COOH (Kraemer et al., 2019). In the course of these intoxications, the initially observed symptoms included insomnia, mydriasis, tachycardia, and lack of response to pain stimuli.

Marinetti and Antonides detected MDPV, α-PVP, methylone, pentylone, and pyrovalerone in 23 cases. Except for one case, where only MDPV was determined, there were also other synthetic cathinones, classic drugs, or medications (Marinetti and Antonides, 2013). In 12 cases, the cause of death was determined as mixed intoxication with detected xenobiotics. MDPV was detected in nine cases, and in two of those cases MDPV intoxication was the main cause of death. Alpha-PVP itself together with other substances was present in two cases and in one case pentylone and alpha-PVP were found in combination with other xenobiotics. Unfortunately, the concentrations of alpha-PVP and pentylone were not determined. In two cases of MDPV intoxication, the identified concentrations of this substance were 91 ng/ml in femoral blood, 132 ng/ml in eyeball fluid, and > 200 ng/ml in urine (in the first case) and 4,800 ng/g in liver (in the second case). It is difficult to unambiguously interpret the above MDPV concentrations due to the presence of other xenobiotics, which is why cases of simple intoxications are most valuable. Of the remaining 11 cases, where another cause of death was identified, MDPV was the only identified substance in one case. MDPV concentrations were 640 ng/ml in femoral blood, 896 ng/g in brain, 6080 ng/g in liver, 1,880 ng/g in bile, and 940 ng/g in eyeball fluid. The cause of death in this case was suicidal hanging. These results indicate that the above MDPV distribution in the body was toxic but not lethal.

Similar conclusions can be drawn from case studies where deaths were caused by accidents. In one case, MDPV concentration in heart blood was 56 ng/ml, while for methylone it was 729 ng/ml. In another case, pyrovalerone concentration was 42 ng/ml in femoral blood, 59 ng/ml in heart blood, 48 ng/g in brain, 124 ng/g in liver, 1,880 ng/ml in bile, and 24 ng/ml in eyeball fluid. Additionally, in the last of the above cases, pentylone was found in blood, but only qualitative analysis was made (Marinetti and Antonides, 2013).

These results show that distribution of xenobiotics in the body can be toxic but not lethal. Knowledge about the distribution of synthetic cathinones in various body tissues can facilitate the understanding of the mechanisms of their toxicity. However, such data are still rare in professional literature (Vignali et al., 2019; Wyman et al., 2013).

7. Summary

Despite the tightening and changing legal regulations concerning production, processing, selling, and possessing substances from the group of synthetic cathinones, abuse of these compounds for recreational purposes still remains at a high level. Although the first synthetic cathinones were synthesized in the 1920s, a rapid increase in the popularity of these substances on the illicit drug market has been recorded after the year 2000 and is probably fuelled by the legality of these substances. Synthetic cathinones are still very popular despite the lack of detailed scientific research on the harmful effects of these substances on the human body. Basic problems related to NPSs mainly concern their widespread availability and administration together with other xenobiotics, the large number of such substances, and dynamic qualitative and quantitative changes on the drug market.

The chemical structure and analytically confirmed cases of intoxication with synthetic cathinones point to the similarity of action (clinical effects) between these compounds and amphetamine or MDMA. Synthetic cathinones exert toxic effects not only on the CNS but also on the cardiovascular system, gastrointestinal tract, or kidneys. Chronic intake of high doses of synthetic cathinones causes development of tolerance, addiction, and, in the case of discontinuation, withdrawal symptoms. In extreme cases, these substances may also cause multi-organ failure leading to death. Numerous deaths have been reported in association with the use of synthetic cathinones. The use of synthetic cathinones should be considered a serious threat to health and life and intoxications with the most commonly used compounds should be monitored by clinical toxicology units and forensic facilities. There is little data on the pharmacodynamics and pharmacokinetics of synthetic cathinones in people, and the current understanding of the problem is mainly based on a small number of *in vitro* and animal studies. Further research is needed on the mechanisms of action, toxicokinetics, toxicodynamics, metabolism, clinical effects, and addictive potential of synthetic cathinones, especially in the context of potential risks caused by increased consumption of this group of drugs in future.

Author contributions

Piotr Czekaj, Ewelina Pieprzyca, Rafał Skowronek: concept, desig, review & editing.

Ewelina Pieprzyca, Rafał Skowronek, Ľuboš Nižnanský and Piotr Czekaj: writing original draft.

Declaration of competing interest

The authors declare no conflict of interest.

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