

Piperazine derivatives as dangerous abused compounds

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Piperazine derivatives are a group of compounds with a psychostimulant effect. They are an alternative to illegal drugs. They are being searched for recreational use due to their psychoactive and hallucinogenic effects. The high popularity of these compounds can be noticed all over the world due to easy purchase, lack of legal regulations and incorrect assessment of the safety of use. The recreational use of piperazine derivatives can often result in chronic and acute health problems and additionally with unpredictable remote effects. It is also common to take mixtures of psychoactive compounds. This hinders the correct diagnosis and treatment of patients with poisoning. The presented work is an illustration of the wide problem of piperazine derivatives abuse. The health effects and the possibility of identifying these compounds in preparations and biological material are described.

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INTRODUCTION

Available literature data indicates a growing interest in new psychoactive substances (NPS) around the world (1, 2). These products, often called designer drugs, can be divided into three main groups: piperazine derivatives, ketoarylamines and synthetic cannabinoids. The modified chemical structures of these compounds aim to mimic the pharmacological effects of illicit drugs and trigger potential abuses. The problem of today is consumers experimenting with these substances in order to achieve a specific benefit (3, 4). Access to NPS is easy and without an atmosphere of legal sanctions. Designer drugs are characterized by a diverse composition and are often combined with other mixtures and forms of psychoactive compounds (5, 6).

Piperazine derivatives can be divided into two classes: benzylpiperazine derivatives and phenylpiperazine derivatives (1, 4, 7). In previous years, studies were undertaken to clarify the properties of piperazine compounds. In the 70th and 80th benzylpiperazine and its derivatives were repeatedly tested as antidepressants. They did not find a medical application because of their amphetamine-like effects, even though they were less intense. Some piperazine derivatives have been identified as active metabolites of the therapeutic

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drugs (7, 8). For example, mCPP [1-(3-chlorophenyl)piperazine] is a metabolite of psychotropic drugs such as trazodone, nefazodone, etoperidone and mepiprazole. MDBP [1-(3,4-methylenedioxybenzyl)piperazine] is the major metabolite of therapeutic drug fipexide, which has been withdrawn due to adverse reactions, including liver toxicity. Another MeOPP [1-(4-methoxyphenyl)piperazine], phenylpiperazine derivative, was used in an *in vivo* study with Wistar rats (7). It was metabolised to *N*-acetyl-4-hydroxyaniline which corresponds to analgesic acetaminophen (paracetamol). The characteristic ring of piperazine is also found in the structure of various therapeutic drugs, for example, clozapine, imatinib, trimetazidine (9–14). For these therapeutic drugs, therapeutic efficacy and undesired events are monitored, and the analytical methods that are developed serve qualitative and quantitative determination. Recent studies indicate a great interest in compounds containing the ring of piperazine (15). The relationships between the chemical structure of piperazine derivatives and effects on the body are analysed.

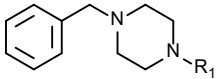
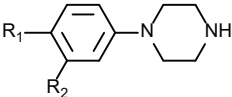
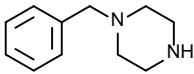
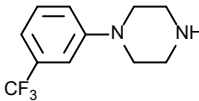
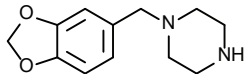
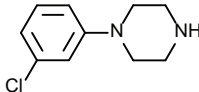
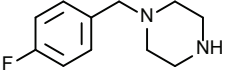
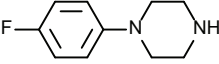
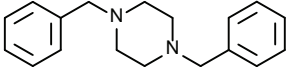
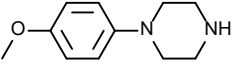
Piperazine derivatives are sought by users for recreational purposes due to their psychoactive and hallucinogenic effects, unusual perception and experience after ingestion (7). The use of piperazine derivatives may result in the development of serious health problems. There are known cases of hospitalizations due to adverse events. Even a single use can lead to dangerous poisoning or death (8). Although many studies have been carried out, toxicokinetic and toxicodynamic knowledge is still limited. In many hospitals, the majority of patients do not have analytical confirmation of poisoning (7, 16). The most important is the correct identification of these substances in biological and non-biological matrices, proper diagnostics of patients and effective medical help (7, 17, 18). The abuse of piperazine derivatives is dangerous especially because these substances can be freely sold in many countries and on the Internet.

The presented work is an illustration of the modern problem of the abuse of piperazine designer drugs and serious health consequences resulting from it. The possibilities of using the latest technologies to analyse these substances in preparations and biological material are presented. This review also shows the striving of scientists to fully understand the effects of piperazine compounds on the human body. The collected information can be the basis for further, targeted research.

PIPERAZINE DERIVATIVES IN DESIGNER DRUGS

Considering the chemical structure, piperazine compounds are derived from piperazine, a cyclic molecule with two nitrogens in the opposite position and four carbon atoms occurring between the two nitrogen atoms (7, 19, 20). The most popular piperazine derivatives present in designer drugs are benzylpiperazine derivatives, *e.g.*, *N*-benzylpiperazine (BZP), 1-(3,4-methylenedioxybenzyl)piperazine (MDBP), 1-(4-fluorobenzyl)piperazine (pFBP), 1,4-dibenzylpiperazine (DBZP) and phenylpiperazine derivatives, *e.g.*, 1-(3-trifluoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP), 1-(4-parafluorophenyl)piperazine (pFPP) and 1-(4-methoxyphenyl)piperazine (MeOPP). Table I presents the structural formulas of piperazine and the most popular piperazine derivatives occurring in designer drugs. Other derivatives of benzyl and phenylpiperazine, which may be components of preparations of designer drugs are: 1-(2,3-dichlorophenyl)piperazine (2,3-DCPP), 1-(4-bromo-2,5-dimethoxybenzyl)piperazine (2C-B-BZP), 1-benzyl-4-methylpiperazine

Table I. The most popular piperazine derivatives occurring in designer drugs

Benzylpiperazine derivatives		Phenylpiperazine derivatives	
			
BZP	<i>N</i> -benzylpiperazine	TFMPP	1-(3-trifluoromethylphenyl)piperazine
			
MDBP	1-(3,4-methylenedioxybenzyl)piperazine	mCPP	1-(3-chlorophenyl)piperazine
			
pFBP	1-(4-fluorobenzyl)piperazine	pFPP	1-(4-parafluorophenyl)piperazine
			
DBZP	1,4-dibenzylpiperazine	MeOPP	1-(4-methoxyphenyl)piperazine
			

(MBZP), 1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine (mCPCPP), 1-(3-methylbenzyl)piperazine (MeBP) and 1-methyl-3-phenylpiperazine (MePP).

The most common names of preparations containing piperazine derivatives are: Frenzy, Legal X, Legal E, Bliss, A2, Rapture, Charge and Herbal Ecstasy; other common trade names are: Benny Bear, Flying Angel, Twisted, Jax, Nemesis and Red Eye Frog (7, 21, 22). While browsing Internet forums, you can still find various other names, forms and doses of preparations containing piperazine derivatives. Some products containing pFPP (also known as fluoperazine, flipiperazine, 4-FPP), are called: The Big Grin, Mashed, Extreme Beans, Playboy Bunny Tablets, Retro pills, Lab-X. Offered doses range from 20 to 150 mg. The sophisticated preparations under the name Cherries contained pFPP and TFMPP. Products containing mCPP were found under the names: Arlequin, X4, Rolls Royce or Smarties (23). Depending on the quantitative combination of BZP and TFMPP, the following product names can be listed: Bliss, Clear Light, Combo, Exodus and The Good Stuff (24). A combination of up to four different piperazine derivatives was also found, which had the names: Party Pills, PEP and X4 (5). Tablets with mCPP can have various colours and logos, *e.g.*, Mitsubishi, Lacoste (8).

Piperazine derivatives in designer drugs are often found together with other psychoactive substances, for example, ecstasy, cocaine, amphetamine, ketamine, cannabis (5, 25).

They can also be included in tablets sold to consumers as ecstasy or amphetamine. There have also been found alcohol solutions of piperazines and mixtures of piperazines in various quantitative proportions with derivatives of cathinones and synthetic cannabinoids (2, 23). Amphetamines may increase the stimulatory effect of BZP (2). Simultaneous alcohol consumption may be associated with the sobering effects caused by piperazine derivatives. The negative effects of using other party pills can be well tolerated with the simultaneous use of BZP. Taking BZP with cannabis derivative is likely to induce users to relax, stimulate appetite and improve sleep. They are often used together with BZP: caffeine, herbal extracts, electrolyte mixtures and amino acids. For example, L-tyrosine, which is a precursor of dopamine, can be taken to counteract the depletion of dopamine.

The usual doses of BZP are in the range of 50 to 250 mg (26). They result in effects lasting from 6 to 8 hours. It has been observed that the onset of BZP activity may be delayed up to 2 hours. This may be the cause of an increased risk of an overdose of this compound due to users taking multiple doses before the onset of effect. Cohen and Butler (27) in their study draw attention to the analytically identified level of BZP in party pills which was between 28 and 133 mg, with an average level of 65 mg per tablet. They also indicate reports on higher levels of BZP up to 1000 mg. They suggest that BZP could be detected in the blood up to 30 hours after ingestion. They also noted that BZP in party tablets often occurs in combination with TFMPP in a ratio of 2:1 to 10:1. The quantitative proportions were 100 mg BZP and 30 mg or 50 mg TFMPP, and 80 mg BZP and 40 mg TFMPP (24, 28). The mCPP content in the example of the analysed tablet was 45.8 mg (8).

The components of designer drugs are often contaminants or substances added to interfere with rapid diagnostic tests (29). These are easily publicly available products, among which can be mentioned: bleach, table salt, detergents, vinegar, lemon juice, hydrogen peroxide and many other commonly harmful substances. Ingredients may also include medicinal products containing psychoactive substances (30). Among such active ingredients are dextromethorphan (codeine substitute), benzydamine, ephedrine and pseudoephedrine. New psychoactive substances may also contain natural products such as plant fragments, cacti and animal venom (31).

There is currently a very large number of products containing piperazine derivatives on the market. The qualitative and quantitative composition of designer drugs changes constantly to avoid legal verification. In addition, it is possible to modify the chemical structures of the compounds presented above. All that gives access to creating hundreds of new products with potential psychoactive effects. Therefore, it is necessary to monitor the global market in the case of designer drugs. According to the European Drug Report (32), piperazine derivatives are on the list of products being subjected to frequent confiscation. Piperazine derivatives are compounds with high potency and pose a serious threat to public health.

CONSUMER'S PROFILE OF PIPERAZINE DESIGNER DRUGS

The formulations containing piperazine derivatives are most often sold as party pills (5, 27). They are offered for sale also in the form of powder, capsules, tablets, smoking forms, liquid mixtures and injections (1). They produce stimulating and hallucinogenic effects and they are designed to imitate the effects of ecstasy (1, 18, 27). Abuse of party pills usually takes place in pubs, bars, at dance parties or rave music concerts, on the beach, in the park

or simply in the street (27). Synthetic substances, together with the desired sensations, can also create a number of unwanted and sometimes unsafe effects on consumers (1).

Ren *et al.* (18) noticed that, despite economic growth, there was some serious malaise, especially among young people and children. They emphasized that the younger generation is dependent on new stimulants of TFMPP, mCPP and other piperazine designer drugs. In recent years, an increase in the abuse of piperazines has been reported in New Zealand, America and Japan. Lack of legal regulations and the susceptibility of young people to taking such compounds may lead to an uncontrolled threat of spreading new psychoactive substances.

Schifano *et al.* (4) stressed that the growing number of stimulants and parallel changes in the patterns of their use pose a challenge for psychiatry. People are susceptible to taking these substances to improve the performance of the body, improve the image, the intensity of psychoactive activities and the probable lack of detection in routine screening. It was also suggested that the increase of interest in a particular psychoactive compound is preceded by the occurrence of clinical events at the level of population. The role of the Internet in shaping the supply market and exchange of experience was underlined (33, 34). The problem with consumers of designer drugs is that they take the risk of experimenting with new substances (3). That decision is made in order to achieve a specific benefit: a state of pleasure, relaxation, unreality or forgetfulness. It can also be a way to reduce pain and suppress feelings of guilt, suppress anxiety and unpleasant memories. The attitude of society is focused on quick methods of acting, which is why young people often reach for quick and risky solutions. An additional problem is the lack of knowledge of the actual chemical components contained in psychostimulants. There is often a lack of information on metabolism, addiction potential and serious adverse effects resulting from unexplored acute and chronic NPS toxicity (35).

The abuse of BZP was also observed among students as well as shift workers and truck drivers in order to increase vigilance, improve physical and mental fitness (2). It was used as an appetite suppressant as well as in the sports to improve performance (36).

In New Zealand, a population survey was carried out to assess the impact of BZP banning. Wilkins and Sweetsur (37) presented a comparison between the levels of use BZP both before and after the introduction of the ban. They also analysed the reasons why users decided to stop using BZP. In addition to health concerns, the individual decisions related to the use of psychoactive substances are influenced by youth and culture trends, changes in accessibility and prices, changes in legal regulations, stringent controls and a targeted law enforcement campaign. It was observed that unpleasant side effects and the ban introduction led to a decline in the use of BZP in New Zealand.

The latest technological advances, easy access to various information *via* the Internet and the development of trade contribute to the growing popularity of branded drugs. On the other hand, everyday life brings a high pace of life, nervousness and quick pursuit of the goal. Also, people are constantly following new products and fashion, which reduces vigilance for the risks associated with NPS. The search for positive mood effects such as feelings of relaxation and pleasure is the main reason for using piperazine designer drugs. The more people succumb to such a habit, the stronger their dependence on psychostimulants becomes. The potential of NPS to cause the damage may also result from the lack of experience of users in the field of, *e.g.*, dosage. Lack of specific knowledge about the effects of psychostimulants is also a cause of many health and social problems.

PHARMACOLOGICAL PROFILES OF PIPERAZINE DERIVATIVES

In the pharmacological profile studies, piperazine derivatives were evaluated as compounds leading to an increase in dopamine (DA), serotonin (5-HT) and noradrenaline (NA) levels (1). New psychoactive substances can cause the release of monoamines through exocytosis and the process of non-exocytosis (38). In addition, they may increase the level of monoamines by inhibiting reuptake, reversing the transporter stream, blocking the transporter, and agonistic activity in postsynaptic receptor sites (38, 39). The influence of piperazine derivatives on DAT transporters (dopamine reuptake transporter), SERT (serotonin reuptake transporter) and NET (norepinephrine reuptake transporter) was analysed (38). Increased levels of DA, 5-HT and NA neurotransmitters can cause desirable as well as adverse behavioural and clinical effects. Increased level of dopamine is the cause of strengthening and behavioural-stimulating effects (40). Elevated levels of norepinephrine can have effects on the cardiovascular system (tachycardia, hypertension) (38). Analogously, an increased level of serotonin can be the cause of entactogenic effects and life-threatening serotonin syndrome (38, 41). In addition, every mood-changing compound used has addiction potential (42). Among the piperazine derivatives in use, BZP is one of the most common and the most studied compounds (7, 43). In the range of dopaminergic BZP activity, observed are motion disorders, hypertension, tremors, vomiting, urinary retention, paranoid psychosis. The adrenergic effects of BZP include symptoms such as anxiety, dilated pupils, insomnia, dizziness, hyperventilation, respiratory acidosis, palpitations, tachycardia, chest pain, seizures, hypertension and ischaemia (2, 7). From the cholinergic side, after the use of BZP, the following symptoms are observed: xerostomia and vomiting, and the emerging immunological disorders are hypersensitivity reactions and pruritus (2). The serotonergic activity of BZP shows effects like agitation, confusion, dissociation states, headache, hangover symptoms, nausea, vomiting, abdominal pain.

BZP binds to 5-HT_{1A}-D and 5-HT_{2A}-C receptors at the micromolar level (38). It strongly inhibits monoamine reuptake by NET whereas low BZP concentrations induce monoamine release by DAT and NET. It inhibits DA reuptake and stimulates NA release for amphetamine-like effects (43). Katz *et al.* (2) pointed out that BZP inhibits SERT serotonin transporters and binds as an agonist to 5-HT₁ receptors, and in high doses binds to the 5-HT₂ receptor causing a mild hallucinogenic effect. Orsolini *et al.* (44) noted that the hallucinogenic properties of NPS occur through binding to the 5-HT_{2A} receptor. The binding of BZP to 5-HT_{2B} causes gastrointestinal effects: abdominal pain and nausea, whereas 5-HT₃ induction causes the onset of migraine (7). In the rat brain synaptosomes, BZP strongly inhibited DA and NA reuptake, had little effect on 5-HT reuptake, stimulated DA, NA release and almost no effect on 5-HT release.

TFMPP and mCPP bind most strongly to serotonin 5-HT_{1A}-D and 5-HT_{2A}-C receptors and induce monoamines release by SERT (38). The TFMPP binds selectively to 5-HT₁ and 5-HT₂ (43). The secretion of hormones such as ACTH, cortisol and prolactin are also mediated by 5-HT receptors (7). The mCPP strongly inhibits monoamine reuptake *via* SERT (38). In the rat brain, it strongly stimulated the release of 5-HT, DA and NA (7). The effect of mCPP as an agonist on 5-HT_{2C} receptors is associated with decreased appetite (45, 46). Activation of 5-HT₃ receptors may be the cause of nausea induced by mCPP (7). The mCPP and MeOPP together strongly inhibited monoamine reuptake. In addition, MeOPP had quite high monoamine release activity.

The combination of BZP with TFMPP or mCPP can mimic the ecstasy profile because BZP releases DA, and TFMPP and mCPP are serotonergic agonists (47). The combination

Table II. Concentrations of selected piperazine derivatives determined in human serum and estimated in human brain after recreational use (38)

Group	Compound	Range of serum concentrations ($\mu\text{mol L}^{-1}$)	Range of estimated concentrations in the brain ($\mu\text{mol L}^{-1}$)
Piperazine derivatives	BZP	0.2–36	0.2–36
	TFMPP	0.3–1.2	22–89
	mCPP	0.1–1.6	1.7–85

BZP – *N*-benzylpiperazine, mCPP – 1-(3-chlorophenyl)piperazine, TFMPP – 1-(3-trifluoromethylphenyl)piperazine

of BZP and TFMPP raises DA and 5-HT concentrations similarly to ecstasy. The mCPP compared to MDMA has lower efficacy as a DAT and NET inhibitor and lower induction of 5-HT release.

Hondebrink *et al.* (38) have analysed the available data on the neuropharmacological and neurotoxicological effects of the large number of NPSs. Researchers pointed out that clinical effects should be correlated with estimated concentrations in the human brain. For most of the substances, the estimated concentrations in the brain exceed levels in the blood. There may be differences of up to several orders of magnitude for different NPSs. In the case of the analysed piperazine derivatives, it was noticed that the range of serum concentrations and estimated brain concentrations are comparable for BZP, while there are differences for TFMPP and mCPP. A summary of these data is presented in Table II.

Luethi and Liechti (48) examined *in vitro* the pharmacological profiles of new psychoactive substances that interact with monoaminergic systems. Researchers used important pharmacological data and estimated doses reported by recreational users and doses available from clinical trials for analysis. The proposed dosage from clinical trials was 100 mg for BZP (fixed dose), and 0.5–0.75 mg kg⁻¹ for mCPP. It was found that the inhibition of norepinephrine transporter (NET) and dopamine transporter (DAT) was potentially strongly correlated with human doses of stimulants used. It is reported that the values of these defined inhibitory strengths may be a marker of substance psychoactivity in humans and may be useful as predictors for human doses. An additional indicator in such results may be the SERT inhibitory potential (serotonin transporter).

Kirla *et al.* (49) have evaluated toxicity, toxicokinetics and behavioral effects of meta chlorophenylpiperazine (mCPP) in zebrafish larvae. They showed higher mCPP toxicity compared to cocaine and a negative effect on larvae development. They highlighted the similarities and differences between mammal and fish models. They presented the utility of zebrafish larvae as a model for screening new psychoactive substances.

In summary, homeostasis is a fundamental index of psychological balance and general health. New psychoactive substances, including piperazine derivatives, affect many different neurotransmitter systems. They cause mood changes, psychological and psychobiological disorders. The biggest problem is that after a short period of mood increase and increased body activity, there is a period of neurochemical exhaustion and a serious health problem may develop. Therefore, the desire to understand the full effect of piperazine compounds on the human body is fully justified. When NPS enters the drug market, both positive and

negative effects are unknown at first. That is why it is important to gather information on the structure of psychoactive compounds, effects on the body and selectively differentiate the symptoms they cause.

THE EFFECTS OF ABUSE AND HEALTH CONSEQUENCES

Arbo *et al.* (50) have shown toxic effects of piperazine derivatives against cardiac cells. Four compounds were tested: BZP, TFMPP, MeOPP and MDBP among which the TFMPP had the strongest cytotoxic activity. The studies were conducted using the H9c2 cell line derived from the heart of the rat. In the heart, piperazine derivatives induced cell death by disturbing Ca^{2+} homeostasis, ATP depletion and significant loss of mitochondrial membrane potential. A significant reduction in intracellular ATP is a hallmark of hypoxia and toxic damage. It was noted that mitochondria play an important role in the induction of cardiotoxicity by piperazine derivatives. Loss of mitochondrial membrane potential impairs oxidation, phosphorylation, destroys energy and induces cell death. Disorders in Ca^{2+} homeostasis can cause serious heart rhythm disturbances. The high level of mitochondrial Ca^{2+} triggers the mitochondrial permeability transition pore (MPTP), leads to the collapse of mitochondrial membrane potential, discontinuation of ATP production and cell death. A neutral red (NR) uptake assay was performed to confirm the results. This test is based on the ability of living cells to bind a weak cationic dye. All piperazine designer drugs caused concentration-dependent cytotoxic effects. Repetto *et al.* (51) in their study described neutral red uptake assay as one of the most commonly used cytotoxicity tests for primary cells and cell lines from various sources. The researchers' justification of the cardiotoxic effects of piperazine derivatives at the cellular level indicates a high risk of exposure to the health and lives of potential consumers (50). Due to the large number of NPS on the drug market, rapid cardiotoxicity screening of emerging NPS is necessary (52).

Persona *et al.* (21) showed the toxic action of BZP on the mitochondrial level in the human glial cell line population LN-18. They presented a significant increase in LDH (lactate dehydrogenase), which is a negative prognostic factor. These changes led to excessive production of reactive oxygen species, oxidation of lipids, proteins or DNA, and reduction of ATP levels in cells. Excessive production of ROS (reactive oxygen species) caused hyperthermia. The DNA damage has been demonstrated by analysing the level of the 8-OHdG (8-hydroxy-2'-deoxyguanosine) marker. In their results, the researchers presented the effect of BZP on an increase in caspase-3 activity, which may be a factor inducing cell death. It was noted that BZP at the highest concentration caused the activation of caspase-9 related to the mitochondrial pathway. It did not affect significantly the activation of caspase-8 related to the receptor pathway. These findings indicate that BZP can affect the induction of the mitochondrial pathway of apoptosis in glial cells. Researchers have shown that organs in which cells have a large number of mitochondria are particularly susceptible to the harmful effects of BZP.

Functional magnetic resonance imaging (fMRI) is a technique used in humans to detect the effects of BZP and TFMPP on neuronal activation, receptors stimulation and the release of neurotransmitters (2, 53). Studies have shown increased activation of the mesolimbic system in the nervous system in response to anticipation of rewarding stimuli. Curley *et al.* (53) examined the effects of BZP and TFMPP given separately and in combination at the expected stage of processing in the reward system. In the brain regions related to people's reactions to risk and uncertainty changes were observed. The BZP dopaminergic effects seemed

to increase the positive agitation and then reduce the reaction to uncertainty whereas TFMPP seemed to increase the emotional response.

Researchers from New Zealand have investigated the impact of BZP and TFMPP on psychophysiological parameters of information processing in healthy male volunteers (28). They showed a negative impact on sensory processing. The study was conducted using electroencephalography (EEG), analysing the brain's bioelectrical activity. In the applied test, the potentials evoked by the specific event (event-related potentials, ERP) were measured (28, 54). Changes in the electric voltage in the brain that occur at the moment of the stimulus were analysed. Lee *et al.* (28) have shown the disruption of the nerve processes required to respond to stimuli, such as attention, memory update and auditory information processing. Researchers concluded that the observed changes may be related to 5-HT, DA and NA transmission disorders caused by the tested compounds.

Zwartsen *et al.* (22) have examined the effect of piperazine derivatives on spontaneous neuronal activity in rats using microelectrode matrices (MEA). All test substances inhibited neuronal activity depending on the concentration. The possibility of separate structure-activity relationships (SAR) was indicated, based on the observed wide range of IC_{50} (inhibitory concentration). The effects of substances belonging to the same chemical group were compared. Among piperazines, the difference between BZP (IC_{50} values $161 \mu\text{mol L}^{-1}$) and TFMPP and mCPP (IC_{50} values of 19 and $32 \mu\text{mol L}^{-1}$, resp.) was reported. This difference was most likely caused by the addition of a halogen moiety on the phenyl ring, which increased the binding to target macromolecules. The authors showed that these studies could be the first step in combining chemical structures and substance classes in order to determine the structure-activity relationship (SAR) even before NPS appears on the market.

Dias-da-Silva *et al.* (55) have conducted an assessment of the hepatotoxic effects of piperazine derivatives BZP, TFMPP, MeOPP and MDBP. The authors became interested in hepatocytes because of their function in the detoxification process. Researchers analysed two human liver cell lines (HepaRG and HepG2) and primary rat hepatocytes. They showed the harmful effects depending on the type of piperazine derivative and its concentration. The strongest cytotoxic activity was caused by TFMPP. For all piperazine derivatives tested, it could be observed: intracellular GSH (glutathione) depletion, ATP depletion, oxidative stress, loss of mitochondrial membrane potential, caspase-3 activation and cell death. Among other things, the authors noted the induced intracellular depletion of GSH caused by piperazine designer drugs. The GSSG (glutathione disulphide) formed was moved into the extracellular medium to protect cells from oxidative stress. This type of reaction was compared with the reactions to amphetamine and MDMA. Similarities have been demonstrated between these metabolic pathways and the pathways described for piperazines. Gu *et al.* (56) confirmed in their research a previously reported mechanism regarding glutathione (GSH) detoxification for piperazine bioactivation products. They have analysed unusual re-arranged Cys-piperazine (cysteine-piperazine) and Gly-Cys-piperazine (glycine-cysteine-piperazine) adducts in rat and human liver microsomes. Researchers accepted a mechanism for bioactivation of the piperazine ring to obtain an imidazoline derivative. In summary, these authors have pointed out that the examined metabolic pathway appeared to be a common GSH detoxification mechanism for piperazine bioactivation products.

Recreational usage of products with psychoactive effects have become popular among young people. Min *et al.* (57) noted the risk associated with hormonal disorders and reproductive functions. Their research showed the estrogenic activity of BZP and TFMPP, which

Table III. List of health consequences of abuse of piperazine derivatives

	Adverse effects of abuse piperazine derivatives	Reference(s)
Psychiatric disorders	Agitation, anxiety, confusion	1, 2, 7, 27, 38, 43
	Insomnia, feelings of breakdown	1, 2, 7, 26, 27
	Fatigue, drowsiness	1, 2
	Lowered mood, tension, anxiety	2, 7
	Anxiety, paranoia, depression	1, 7, 26, 43
	Euphoria/dysphoria	4, 19
	Mood swings, panic, bad mood	25, 27, 37
	Hallucinations, especially auditory hallucinations	1, 7, 26, 38
	Withdrawal from social interactions	1
	Dissociative symptoms	2, 7, 25
Cardiovascular	Tachycardia	1, 2, 7, 19, 25, 26
	Increase in systolic and diastolic blood pressure	1, 2, 4, 7, 19
	Palpitation, circulatory collapse	2, 7, 26, 27, 43
	Myocardial infarction	2, 50, 52
	Chest pain	1, 2, 7, 43
	Prolongation of the QT interval	1, 26
Neurological	Headaches and dizziness	1, 2, 7, 26, 27, 43
	Migraine	2, 7
	Pupils dilation	1, 2, 7, 25
	Blurred vision	7
	Convulsions, trembling, shaking the body	1, 2, 26, 27, 43
	Epileptic seizures, repeatability of epileptic seizures	1, 2, 4, 5, 7, 27, 43
	Increased sensitivity towards light and noise	7
	Poor concentration	27
Metabolic	Hyponatremia	1, 4, 43
	Metabolic and respiratory acidosis	1, 2, 43
	Increases in plasma prolactin, cortisol and ACTH	7
Gastrointestinal	Nausea and vomiting	1, 2, 7, 25, 26, 27, 43
	Lack of appetite	2, 45, 46
	Dehydration	2
	Abdominal pain	2, 7, 37
Kidney	Urinary retention	2, 7, 43
	Renal failure, acute kidney injury	1, 2, 4, 7
Pulmonary	Hyperventilation	2, 7, 43
Muscle	Rhabdomyolysis	1, 2
	Muscle contractions	25

Skin	Reddening	7
	Pruritus	2
Other	Xerostomia	2
	Hyperthermia	1, 2, 7, 43
	Abundant sweating, chills	1, 7, 26, 43
	Bruxism, problems with teeth and jaw	1, 7, 25
Heavy complications	Intravascular coagulation	1, 2
	Multiple organ failure	1, 2, 42
	Serotonin syndrome	1, 2, 4, 7, 26, 38, 41
	Hepatotoxicity	2, 7, 55
	Attempts to commit suicide	1
	Nephrotoxicity requiring dialysis	1
	Autoimmune diseases	21
	Malignant neoplasms	21
Fatal cases	1, 7, 8, 27	

can be detected at various stages of estrogen signaling pathways from binding to receptors to cellular proliferative responses.

Table III shows a list of various health consequences of the abuse of piperazine derivatives being mentioned in last two chapters (1, 2, 4, 5, 7, 8, 19, 21, 25–27, 37, 38, 41, 43, 45, 46, 50, 52, 55).

In summary, a multidirectional evaluation of the cytotoxic effect of piperazine derivatives was performed. In general, piperazine designer drugs are potentially cardiotoxic, hepatotoxic, neurotoxic, neurodegenerative and they are associated with hormonal disorders. It was emphasized that TFMPP showed the greatest cytotoxicity. The problem is that new psychoactive substances placed on the market are not subjected to any toxicity and teratogenicity tests. Therefore, it is necessary for scientists to do such research. Only by covering all aspects of acute and chronic usage of piperazine derivatives the more complete picture of their damaging effects can be presented.

METHODS OF IDENTIFICATION FOR PIPERAZINE DESIGNER DRUGS

In the analysis of piperazine designer drugs, commonly used are methods based on modern, coupled techniques, especially chromatographic ones (17). Published analytical reports differed in the development of samples and their preparation including hydrolysis of conjugates, extraction of analytes (LLE - liquid-liquid extraction, SPE – solid-phase extraction) and derivatization procedures.

Swortwood *et al.* (58) developed an analytical method using liquid chromatography triple quadrupole-tandem mass spectrometry (LCQQQ-MS/MS). The approved method was successfully used to analyse two *post mortem* cases with suspected use of designer drugs. Among the substances detected and quantified were piperazine derivatives, BZP

and TFMPP. Tang *et al.* (59) carried out the analysis of urine and hair samples, which were collected from patients abusing psychostimulants. The test was performed to approve the locally developed method using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Among the identified substances was also TFMPP. Paul *et al.* (60) have developed a liquid chromatography high-resolution mass spectrometry quadrupole-time-of-flight (LC-HRMS-QToF) method for the urine analysis in order to identify designer drugs and abused drugs. The validation included 39 compounds also related to piperazine derivatives: BZP, mCPP and TFMPP. Concheiro *et al.* (61) have developed a quantitative method targeting 40 novel psychoactive substances, including 8 piperazines and 4 metabolites in urine, using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Boumrah *et al.* (5) have developed a qualitative method using gas chromatography-mass spectrometry, which allows the separation and identification of a mixture consisting of piperazine derivatives and amphetamine-type stimulants. Quantitative analysis was carried out by high-performance liquid chromatography using a liquid chromatograph with a diode-array detector (HPLC-DAD). Gas chromatography-mass spectrometry (GC-MS) is a frequently used technique for the identification and determination of piperazine derivatives and their metabolites in body fluids and in available preparations (17, 62, 63). Beckett *et al.* (64) used the method of isotope ratio mass spectrometry (IRMS) for the determination of the isotope composition of tablets containing BZP and TFMPP. High-performance liquid chromatography (HPLC) was used to separate BZP and TFMPP. Beckett *et al.* (64) showed the possibility of obtaining information on the origin of designer drugs, including BZP and TFMPP by using IRMS. Görgens *et al.* (36) conducted research to identify the highly polar doping compounds used in sports. They developed a method using hydrophilic interaction chromatography coupled with high-resolution mass spectrometry, HILIC-HRMS. Among the polar stimulants, BZP was also identified. DeRuiter *et al.* (65) paid attention to the development of numerous designer drug analogues that are not specifically controlled by existing legislation. In their research, they showed the use of GC-MS (gas chromatography-mass spectrometry) and GC-IR (gas chromatography-infrared spectroscopy) to identify disubstituted piperazine derivatives. Guillou *et al.* (66) have discussed the problem of the responsibility of customs authorities for the control of goods and chemical products entering the European Community market. Recently, Customs authorities reported a large number of NPS being imported from non-European Union countries. The routine control performed by the Customs laboratories mainly includes the use of GC-MS (gas chromatography-mass spectrometry), FT-IR (Fourier transform infrared spectroscopy), NMR (nuclear magnetic resonance), and HR-MS (high-resolution mass spectrometry). They showed that modern analytical techniques and cheminformatics tools make possible the identification of psychoactive substances apprehended by European Customs. They emphasized the great value of the work of Customs laboratories for the protection of public health and the safety of citizens.

A number of other specific methods have been developed for the detection and quantification of analysed compounds. Waite *et al.* (67) have presented chemiluminescence detection of piperazine derivatives using tris(2,2'-bipyridine) ruthenium(III) as a reagent. Selected mechanisms of chemical reactions for usage in rapid tests to addictive substances have already been described in the past (68). Elie *et al.* (69) have developed microcrystalline tests using mercury chloride as a reagent for selected designer drugs, including BZP. In the analysed environment, BZP created characteristic flat and square crystals. Philp *et al.* (70) developed and approved a colour method using the NQS (1,2-naphthoquinone-

4-sulfonate) test reagent to detect piperazine analogs. As a result of the NQS reaction with BZP, a bright, orange-red colour specific only to BZP was developed. Piperazine and four other piperazine derivatives were also tested: TFMPP, mCPP, pCPP, MeOPP, which also showed an orange-red colour. The authors justified the selectivity of NQS reagent and the suitability of the method for the initial detection of piperazine analogues. Castaneto *et al.* (19) have evaluated the efficacy of the Randox BAT (biochip array technology) immunoassay to identify piperazine derivatives in urine samples. Waddell *et al.* (71) developed an electrochemical, voltammetric method of BZP analysis, whereas Bishop *et al.* (72) have optimized a method using capillary electrophoresis with a chiral selector, for the simultaneous separation of amphetamine-like compounds and new stimulants of piperazine derivatives group.

The research was also carried out on the effects of formalin solutions and putrefactive processes on the analysed compounds (73, 74). Johnson *et al.* (75) have evaluated the stability of selected piperazine derivatives in biological matrices under different storage conditions. They showed that storage conditions can have a big impact on getting the right analytical results. Lau *et al.* (76) studied the stability of synthetic piperazines in human blood depending on temperature and storage time. They proved that benzyl piperazines were more stable than phenyl piperazines. The determinations were made using ultra-fast liquid chromatography with Q-trap electrospray ionization tandem mass spectrometer (UFLC-ESI-MS/MS).

In summary, the unambiguous chemical identification of piperazine designer drugs requires the use of advanced analytical techniques. In addition, the tests carried out are time-consuming and the interpretation of the results requires considerable commitment and knowledge. There is no routine analytical approach to piperazine derivatives. That creates a research problem. The methodology for determining piperazine designer drugs in biological material must be constantly developed. The use of methods using modern coupled techniques, especially chromatographic with mass detection, enables comprehensive analysis. By using combined techniques, information can be obtained to defining precisely the structure of the tested compounds. Correct identification and quantification are necessary for the effective diagnosis and therapy of patients with suspected poisoning.

CONCLUSIONS

New psychoactive substances belong to popular, commonly abused compounds. The recreational use of piperazine derivatives can result in acute and chronic health consequences. In addition, it is difficult to predict the behavior of people under the influence of psychostimulants. Problems with understanding and processing information can have a significant impact on one's own and social function and quality of life. Potential users of designer drugs looking for beneficial effects do not pay attention to the simultaneous occurrence of threats. The development of science and technology allows for a new, integrated approach to diagnosis, proper identification of NPSs and effective assistance for poisoning patients. The article presents selected scientific achievements related to piperazine derivatives in designer drugs, obtained in recent years. The presented scientific reports may give the opportunity to analyze the impact of piperazine compounds on the human body. They may be as well the basis for further research aimed at solving the difficulties and threats that these compounds bring to the modern world.

Abbreviations, acronyms, symbols. – 2,3-DCPP – 1-(2,3-dichlorophenyl)piperazine, 2C-B-BZP – 1-(4-bromo-2,5-dimethoxybenzyl)piperazine, 5-HT – serotonin, 5-HT1A-D – serotonin receptors, 5-HT2A-C – serotonin receptors, 5-HT3 – serotonin receptors, 8-OHdG – 8-hydroxy-2'-deoxyguanosine, ACTH – adrenocorticotropic hormone, BAT – biochip array technology, BZP – *N*-benzylpiperazine, Cys-piperazine – cysteine-piperazine, DA – dopamine, DAT – dopamine reuptake transporter, DBZP – 1,4-dibenzylpiperazine, EMCDDA – European Monitoring Centre for Drugs and Drug Addiction, ERP – event related potentials, fMRI – functional magnetic resonance imaging, Gly-Cys-piperazine – glycine-cysteine-piperazine, GSH – glutathione, GSSG – glutathione disulphide, HILIC-HRMS – hydrophilic interaction chromatography coupled with high resolution mass spectrometry, HR-MS – high-resolution mass spectrometry, IRMS – isotope ratio mass spectrometry, LC-HRMS – liquid chromatography coupled to high-resolution mass spectrometry, LC-HRMS-QToF – liquid chromatography high-resolution mass spectrometry quadrupole-time-of-flight, LC-MS/MS – liquid chromatography-tandem mass spectrometry, LCQQQ-MS/MS – liquid chromatography triple quadrupole-tandem mass spectrometry, LDH – lactate dehydrogenase, LLE – liquid-liquid extraction, MBZP – 1-benzyl-4-methylpiperazine, MDBP – 1-(3,4-methylenedioxybenzyl)piperazine, MDMA – 3,4-methylenedioxymetamfetamin (ecstasy), MEA – microelectrode matrices, MeBP – 1-(3-methylbenzyl)piperazine, MeOPP – 1-(4-methoxyphenyl)piperazine, MePP – 1-methyl-3-phenylpiperazine, MPTP – mitochondrial permeability transition pore, mCPCPP – 1-(3-chlorophenyl)-4-(3-chloropropyl)piperazine, mCPP – 1-(3-chlorophenyl)piperazine, NA – noradrenaline, NET – norepinephrine reuptake transporter, NPS – new/novel psychoactive substances, NQS – 1,2-naphthoquinone-4-sulfonate, NR – neutral red, pFBP – 1-(4-fluorobenzyl)piperazine, pFPP – 1-(4-parafluorophenyl)piperazine, SERT – serotonin reuptake transporter, SPE – solid phase extraction, TFMPP – 1-(3-trifluoromethylphenyl)piperazine, UFLC-ESI-MS/MS – ultra-fast liquid chromatograph with Q-trap electrospray ionization tandem mass spectrometer

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