BZP and TFMPP - party pills demonstrating high potential for metabolic interactions with drugs

Benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) are major active components of so called "party pills". Their distribution by funshops and via Internet was legal in Poland until March 2009. Young party participants often perceived BZP/TFMPP formulations as a safer alternative to MDMA and amphetamine due to their "legal status", which frequently led to overdose. BZP owes its stimulatory properties to enhancement of neurotransmission mediated by dopamine and noradrenaline. TFMPP shows affinity for multiple serotonin receptors. The effect of BZP and TFMPP co-administration resembles that of MDMA. Peak plasma concentrations of both substances are reached between 1-2 hours following oral administration. The relatively slow onset of action provokes inexperienced users to repeat initial doses, therefore exposing themselves to higher risk of adverse effects. Tachycardia and elevated blood pressure are characteristic of BZP overdose. So far, all reported cases of death following ingestion of BZP and other piperazine derivatives resulted from poly-drug use. Hepatic metabolism of BZP and TFMPP in humans involves CYP2D6, CYP1A2 and CYP3A4 isoenzymes. Genetic polymorphism of CYP2D6 significantly affects the efficiency of TFMPP oxidation. BZP and TFMPP inhibit each others' metabolism. They are also inhibitors of cytochrome P450 CYP2D6, CYP1A2, CYP3A4, CYP2C9, and CYP2C19 isoenzymes, responsible for the metabolic clearance of most drugs. Therefore, BZP and TFMPP may be involved in a large number of interactions with medicinal drugs, some of them potentially health and life-threatening.

Benzylpiperazyna (BZP) i trifluorometylofenylopiperazyna (TFMPP) są podstawowymi czynnymi składnikami tzw zwanych "party pills". Do marca 2009 r. ich dystrybucja w Polsce odbywała się legalnie w funshopach i poprzez witryny internetowe. Preparaty oparte na BZP i TFMPP, dzięki ich "legalności", często postrzegane były przez młodych uczestników imprez jako bezpieczniejsza alternatywa dla MDMA oraz amfetaminy, co nierzadko skutkowało ich przedawkowym użyciem. BZP zawdzięcza swoje właściwości pobudzającej stymulującej neurotransmisji dopaminowej i noradrenergicznej. TFMPP wykazuje powinowactwo do licznych typów receptorów serotoninowych. Skutki, jakie wywołuje w organizmie stosowanie w połączeniu BZP/TFMPP są podobne do MDMA. Substancje te osiągają maksymalne stężenia w osoczu około 1-2 godzin po podaniu doustnym. Dość późny początek działania stymulantów często skłania mniej doświadczonych użytkowników do przyjmowania zwielokrotnionych dawek, co zwiększa ryzyko wystąpienia działań niepożądanych. Tachykardia i wzrost ciśnienia tętniczego są objawami charakterystycznymi dla przedawkowania BZP. Wszystkie opublikowane do tej pory doświadczenia z powodzeniem wyzwali się z poliprajmagją. BZP i TFMPP ulegają metabolizmowi wątrobowemu przy udziale izoenzymów CYP2D6, CYP1A2 i CYP3A4. Stopień i szybkość oksydacji TFMPP są znacznie modyfikowane przez polimorfizm genetyczny CYP2D6. BZP i TFMPP wzbudzają metaboliczne interakcje z wieloma innych pochodnych piperazyn, niektóre z nich potencjalnie mają wpływ na metabolizm wielu substancji leczniczych, niektóre mogą być powiązane z potencjalnymi ryzykami zdrowotnymi i życiowymi.
Introduction
Piperazine-derived compounds have been in use as recreational drugs since the 1990s [39]. Benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) [Fig. 1] are major active components of so called "party pills", often combined in different proportions.

Other piperazine derivatives found in designer drug formulations include methoxyphenylpiperazine (MeOPP), chlorophenylpiperazine (mCPP), fluoro phenylpiperazine (pFPP), methylbenzylpiperazine (MBZP) and methylenedioxybenzylpiperazine (MBDP).

Unlike most of recreational drugs with a history of medicinal use, these piperazine derivatives have been deliberately synthesized to be marketed as recreational drugs with psychoactive properties [3]. The career of fumshops and websites retailing designer drugs in Poland was spectacular (over 1000 shops opened between October 2008 and October 2010), and so was the famed campaign undertaken by Polish authorities aimed at outlawing them. A rapidly increasing supply of designer drugs and emerging reports of deadly poisonings and hospitalizations related to these substances resulted in strong social response and forced Polish government to take drastic steps. Although the campaign met with general approval and support of Polish society, the legal basis of these actions was questioned by some constitutionalists, human rights activists and drug professionals [1]. Owing to their "legal" status (in Poland until March 2009 [38]) and stimulatory properties, BZP and TFMPP gained significant popularity, predominantly among young party participants, who perceived them as a safer alternative to MDMA and amphetamine [29]. This erroneous belief led to taking doses often several times exceeding those recommended by manufacturers as "safe".

Lack of extensive testing prior to human use, characteristic of medicinal drugs, makes it difficult to predict and to deal with consequences of intoxication with piperazine derivatives. The available data on pharmacokinetic properties of BZP and TFMPP is scant. Both compounds are known to inhibit CYP2D6, CYP1A2, CYP3A4, CYP2C9, CYP2C19, key hepatic cytochrome P450 enzymes responsible for drug metabolism, which may result in numerous, potentially serious interactions with prescription drugs [4].

Dosing
BZP is available mainly in tablet or capsule form [39], with typical doses ranging between 50 and 200 mg per unit [9]. There have been reports of pills containing even up to 1000 mg of BZP [17]. "Party pills" are often made up of a blend of BZP and TFMPP. The BZP to TFMPP dose ratio in such pills usually ranges from 2:1 to 10:1 [38].

Pharmacodynamic properties
BZP demonstrates its effects on monoaminergic neurotransmission mediated by dopamine (DA), serotonin (5-HT) and noradrenaline (NA). The central mechanism of action of BZP in rodents was explained by its interaction with DA, and to a lesser extent, 5-HT transporters. A dose-dependent rise in extracellular DA and 5-HT following intravenous administration of BZP was shown in male Sprague-Dawley rats [7]. Stimulatory effect of BZP on 5-HT, receptors has been described [35], while 5-HT2 receptors were not affected [7]. BZP's stimulant effects result mainly from its cocaine-like inhibition of DA uptake, amphetamine-like dopamine release and agonist activity on postsynaptic DA receptors. Magyar et al. suggested BZP-induced NA release in periphery, which was attributed to α2-adrenergic receptor blocking properties [21]. This rise in peripheral NA results in tachycardia and tachyphylaxis in rats [39]. Benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) demonstrate comparable effect produced by LSD. TFMPP is used as a marker for 5-HT activity due to significant affinity for multiple 5-HT receptor subtypes, for example 5-HT1A, 5-HT1B and 5-HT2 [23]. When co-administered, BZP and TFMPP elevate levels of both DA and 5-HT, similarly to MDMA. Levels of DA and 5-HT released after a combined dose of BZP and TFMPP in rats significantly exceeded the effects of BZP and TFMPP administered separately [7], suggesting pharmacodynamic interaction as an underlying mechanism of this phenomenon.

Pharmacokinetics
The study performed on 7 healthy human volunteers (all male, aged 19-31 years) gave some insight into pharmacokinetic properties of BZP. The peak plasma concentration (Cmax) of 262 ng/mL was reached 75 min (Tmax) after a 200 mg oral dose of BZP. The mean absorption half-life was 6.2 min. The clearance was 58.3 L/h (reported as Cl/F). The elimination half-life was calculated to be 5.5 h. In addition, plasma concentrations of two metabolites were assayed: 4-OH BZP (Cmax of 7 ng/mL at Tmax = 60 min) and 3-OH BZP (Cmax of 13 ng/mL at Tmax = 75 min). Both metabolites were detectable in plasma samples 24h after dose administration. The presence of urinary metabolites such as N-sulfate conjugate of BZP and an O-sulfate conjugate of its hydroxylated metabolites, in addition to 4-OH BZP and 3-OH BZP, was also reported [3].

6 healthy adult volunteers were enrolled in the study on pharmacokinetics of TFMPP. Cmax following a single 60 mg oral dose was 24 ng/mL at Tmax = 90 min. TFMPP had two disposition phases (t1/2 = 2.04 h and t1/2 = 5.95 h). Apparent clearance (Cl/F) was 384 L/h. A single metabolite, 4-OH TFMPP was detected in plasma (Cmax = 20 ng/mL, Tmax = 90 min), and two metabolites: 4-OH TFMPP and an N-glucuronide of TFMPP in urine [5]. The mentioned excretion profile of TFMPP urinary metabolites in humans is consistent with findings of study performed on rats [37].

The experienced "party pill" consumers reported a slow onset of action. Some impatient users, disappointed with lack of expected effect, repeated doses before the Cmax was reached, thereby increasing the risk of toxicity [16].

Adverse effects
Commonly observed adverse effects of BZP and TFMPP include palpitations, agitation, anxiety, confusion, dizziness, headache, tremor, mydriasis, insomnia, urine retention, and vomiting [27]. Tachycardia with elevated systolic and diastolic blood pressure following BZP administration are well documented [8]. Severe adverse effects such as respiratory acidosis, metabolic acidosis, hyponatraemia, toxic seizures, acute renal failure and psychosis have been described [2,17]. There is also a report of rhabdomyolysis, hepatotoxicity, coagulopathy and multorgan failure following BZP ingestion [16]. It is important to note, however, that most of these adverse effects, including death, result from poly-drug use [3]. "Party pills" are often mixed with alcohol, tobacco, MDMA, amphetamine, cannaabis and other illicit as well as medicinal drugs. Therefore, the number of possible interactions and consequential side effects seems unpredictable. Hitherto, the effects than can be unequivocally attributed to BZP alone are stimulant effects such as tachycardia and elevated blood pressure [19] and seizures with higher BZP plasma concentrations [15]. These symptoms may exacerbate or new reactions can occur with concomitant use of other drugs.

Metabolism
The knowledge of metabolic pathways of drugs of abuse is essential in everyday practice of clinical and forensic toxicologists. Metabolites can contribute to some of the toxic effects or may serve as biomarkers of exposure to illicit substances [22]. The metabolism of BZP and TFMPP has been investigated in rats [31] and on human liver microsomes [4]. The experiment performed on rodents showed that 3 isoenzymes of cytochrome P450 are involved in hepatic oxidation of TFMPP: CYP2D6, CYP1A2 and CYP3A4, accounting for 80.9%, 11.5% and...
7.6% of metabolism, respectively. TFMPP particle is subjected to hydroxylation of the phenyl ring and dealkylation of the piperazine ring, followed by degradation, acetylation and conjugation with glucuronate or sulfonate [33]. Due to structural similarity of BZP and TFMPP, analogous pathway with CYP2D6, CYP1A2 and CYP3A4 implicated in BZP metabolism in rats has been proposed.

In the human liver, CYP2D6, CYP1A2 and CYP3A4 have been identified as isoenzymes responsible for BZP and TFMPP metabolism, which is consistent with findings of experiments conducted on animal model [4]. It has been suggested that BZP is excreted mostly unchanged in the urine, while the elimination of TFMPP principally involves the CYP isoenzymes [32, 33].

CYP2D6 is known to be the source of oxidation polymorphism. In up to 14% of Caucasian population, referred to as poor metabolizers, the enzyme is not expressed due to mutations in the CYP2D6 gene on the long arm of chromosome 22. The poor metabolizer status results in compromised metabolism of CYP2D6 metabolites including TFMPP and, to a lesser extent, BZP. In an experiment comparing poor and extensive metabolizers, the pharmacokinetics of BZP was not significantly affected, while Cmax of TFMPP in CYP2D6 poor metabolizers was significantly higher than in extensive metabolizers [3].

Potential for interactions
BZP and TFMPP significantly inhibit each others' metabolism. In an experiment conducted on human liver microsomes, TFMPP inhibited the metabolism of BZP by nearly 60%, and BZP inhibited the metabolism of TFMPP by approximately 91% [4]. Almost 30% of party pills contain a combination of these substances. A metabolic interaction between BZP and TFMPP can lead to elevated levels of both drugs and enhanced effects as well as higher incidence of adverse effects [4]. Taking this into consideration, combining lower doses of BZP and TFMPP in "party pills" compared to formulations containing only one of these agents may reduce adverse effects.

Interactions involving prescription and OTC drugs pose a serious threat to patients and managing them is a challenge for healthcare professionals. Interactions between medicinal and designer drugs are an even more complex problem due to often unknown composition and uncontrolled dosage and analytical statistics. Only six isoenzymes (1A2, 3A4, 2C9, 2C19, 2D6, 2E1) account for the metabolic clearance of more than 90 percent of all drugs [10]. BZP and TFMPP have been shown to inhibit five of them: CYP2D6, CYP1A2, CYP3A4, CYP2C19 and CYP2C9 [4]. Many drugs, often co-ingested with party pills, can be metabolized, which may lead to elevated plasma concentrations and higher incidence of adverse effects.

Using dextromethorphan as a probe substrate, BZP and TFMPP demonstrated inhibitory effects on CYP2D6. Paroxetine, a selective serotonin re-uptake inhibitor, is metabolized by this isoenzyme. Inhibition of paroxetine metabolism can result in an overdose effect leading to the serotonin syndrome, a potentially fatal interaction with symptoms including anxiety, agitation, nausea, diarrhea, shivering, diaphoresis, tremor, hyperreflexia, and autonomic instability (increased or decreased blood pressure and pulse rate). BZP shows serotoninergic activity, which may further contribute to serotonin syndrome through the mechanism of sympathomimetic drug interaction [7].

MDMA is also one of CYP2D6 substrates. This seems crucial in light of the fact that the only cases of death after ingestion of BZP to date were reported to occur with simultaneous consumption of MDMA (ecstasy) [6]. An interaction with metoprolol, a β-blocker metabolized by CYP2D6 and in a small percentage by CYP3A4, is likely to evoke Bradycardia, severe hypotension, diziness, diarrhea and a number of other adverse effects characteristic of β-blockers’ overdose [34].

Caffeine is the ubiquitous stimulant served at bars and clubs all over the world in many alcoholic cocktails and energy drinks. Caffeine is one of the most used and sought-after substances. Tobacco smoking, are known to induce cytochrome P450 2C9 and TFMPP may increase incidence and severity of side effects of benzodiazepines, cyclosporine (immune modulator), macrolide antibiotics (e.g. erythromycin, clarithro-mycin) following CYP3A4 inhibition [14].

CYP2C9 and CYP2C19 are involved in hepatic oxidation of phentyo (antiepileptic) and mepidine (anticoagulant), both characterized by narrow therapeutic index. Cytochrome P450 2C9 isoenzyme metabolizes nonsteroidal anti-inflammatory drugs [14]. Frequent ingestion of BZP/TFMPP pills simultaneously with popular OTC formulations containing ibuprofen or diclofenac may lead to degradation of gastric mucosa and increased risk of ulcer development [11].

On the other hand, the metabolic pathways of BZP and TFMPP may be influenced by different substances. Tobacco smoking is inseparably tied to partying. Polycyclic aromatic hydrocarbons, components of tobacco smoke, are known to induce cytochrome P450 2C19 and TFMPP [13]. Therefore, the elimination of BZP and TFMPP may be accelerated in smokers compared to nonsmokers.

Conclusions
Criminalization of pipazernine derivatives' use and distribution in Poland [24] has undoubtedly contributed to decrease in the number of intoxications caused by these substances. However, the black market trade of BZP and TFMPP still exists and is an option for the most determined party pill users. Thus, more comprehensive pharmacokinetic data on BZP and TFMPP is desirable. Poland is one of the world's leading countries with respect to the amount of consumed OTC drugs. The issue of interactions involving OTC and prescription drugs as well as illicit substances requires further thorough investigation.

References
7. Baumann M., Clark R., Budzynski A. et al. Experimental investigations of the pharmacokinetics of BZP and TFMPP in “party pills” compared to formulations containing only one of these agents. 2009.
of research on benzylpiperazine as a recreational drug. Int. J. Drug Policy. 2011, 22, 2, 95.
11. Dajani E.Z., Agrawal N.M.
18. Kerrigan S., Lindsey T.
23. Miranda F., Orozco G., Velazquez-Martinez D.N.: Full substitution of the discriminative cue of a 5-HT (1A/1B/2C) agonist with the combined administration of a 5-HT (1B/2C) and a 5-HT (1A) agonist. Behav. Pharmacol. 2002, 13, 303.