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Current awareness of piperazines: pharmacology and toxicology

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Although many piperazine derivatives exist, only a limited number have been studied, whereby they have been found to be generally stimulant in nature resulting from dopaminergic, noradrenergic, and predominantly serotoninergic effects in the brain. Reported toxic effects include agitation, anxiety, cardiac symptoms (e.g. tachycardia) and sometimes seizures. As for many drugs, they are primarily metabolized by cytochrome P450 with subsequent possible glucuronidation and/or sulfation. Their abuse has been relatively recently observed in the last decade with only a few identified in biological fluid (primarily 1-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (3-TFMPP)) despite publications of a number of analytical methods. Even when detected, however, the toxicological significance of their presence is often difficult to ascertain as many cases involve other drugs as well as a wide and overlapping range of concentrations found in blood (both in life and after death). This paper reviews the current pharmacological and toxicological information for piperazine derivatives and also includes new ante-mortem and post-mortem blood data.

Keywords: BZP; TFMPP; piperazines

Introduction

The piperazine family of compounds was the first class of abused drugs to follow the numerous phenylethylamine-based compounds (e.g. amphetamine, MDMA, 2C-B).[1] Piperazine itself is widely used as an anthelminthic drug in the treatment of intestinal round worms but the piperazine derivatives have never been used for such a purpose.[2] The piperazine-based compounds are totally synthetic (rather than 'herbal' as purported by some suppliers) and are derived from two main structural groups (1-benzylpiperazine and 1-phenylpiperazine) acting as a framework for substitution. Apart from 1-benzylpiperazine itself (sometimes referred to as simply benzylpiperazine or BZP), other examples include 3-TFMPP (1-(3-trifluoromethylphenyl)piperazine), mCPP (1-(3-chlorophenyl)piperazine), pMeOPP (1-(4-methoxyphenyl)piperazine), pFPP (1-(4-fluorophenyl)piperazine) and MDBZP (or MDBP or 1-(3,4-methylenedioxybenzyl)piperazine) (Figure 1). Due to the possibility of various positional isomers (e.g. 2-TFMPP, 3-TFMPP, and 4-TFMPP) and potential legal consequences, there can be an analytical requirement to discern the specific compound involved.[3] It is also of note that mCPP, for example, is an intermediate substance in the pharmaceutical manufacture of several antidepressants (e.g. trazodone and nefazodone).[4] mCPP is also a predominant metabolite of trazodone and therefore the context of its presence in biological fluid is an important interpretative consideration. They have not been marketed as pharmaceuticals, with only BZP having been evaluated as a potential antidepressant as well as being identified as an active metabolite of the withdrawn antidepressant drug, piberaline.[5]

Abuse history

Abuse was first reported in the late 1990s in the USA and Scandinavia but since 2000, abuse has been reported in many other countries (particularly New Zealand, Australia and across Europe).[1,6,7] The mode of abuse is similar to that of Ecstasy, with many users seeking amphetamine- or MDMA-like effects. Prior to drug control legislation in many countries, many suppliers of piperazines marketed them as 'legal Ecstasy' or as a 'legal high' with products typically containing mixed piperazine derivatives (e.g. BZP and TFMPP) in variable quantities.[8,9] For mCPP, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) indicated that by 2006 it was estimated that almost 10% of illicit tablets sold in the EU, as part of the illicit Ecstasy market, contained mCPP.[10]

The piperazine derivatives are usually found in illicit dosage forms as tablets, capsules or loose powders but rarely liquids. As such, consumption of piperazines is mainly by oral ingestion or insufflation ('snorting') but injection and smoking is possible. The dose can vary between 50 to 200 mg[11] which correlates with dosages used in published trials.[12,13] In a UK study, 20 tablets/capsules contained between 28 and 133 mg of BZP (mean = 65 mg) and 4–72 mg of TFMPP (mean = 22 mg). The stated doses ranged between 105–200 mg BZP and 50–75 mg TFMPP.[9]

Pharmacology

Neuropharmacology

Only a limited number of abused piperazines have been evaluated by researchers, for example, BZP and TFMPP. Overall, studies in animals have demonstrated that they stimulate the release and inhibit the reuptake of dopamine (DA), serotonin (5-HT) and noradrenaline (NA), but dopaminergic and serotonergic effects

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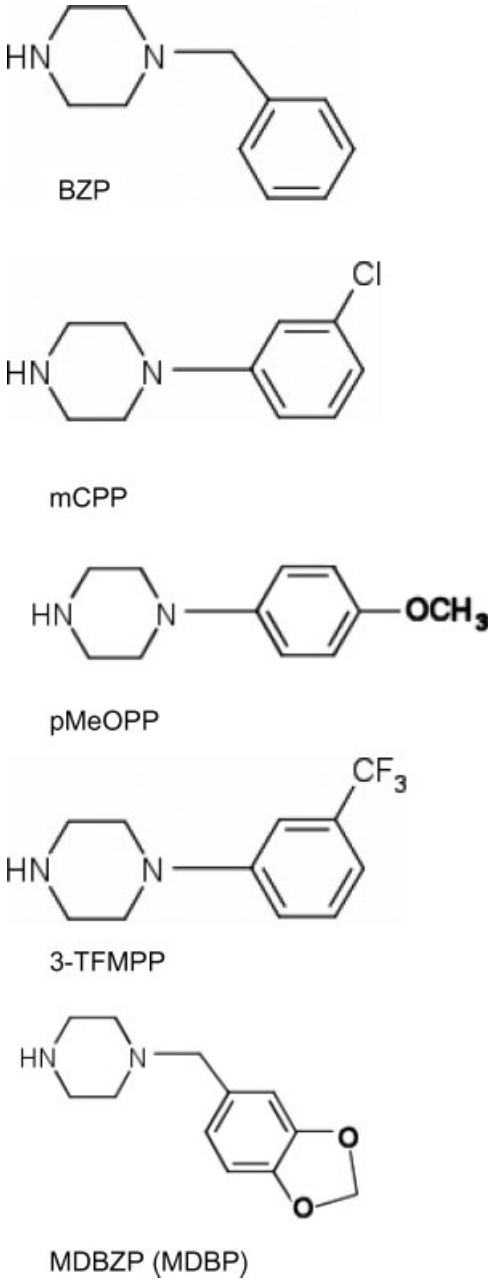

Figure 1. Chemical structure of some abused piperazines.

predominate. During these studies, BZP was found to be less potent than MDMA, methamphetamine or amphetamine.

As part of an assessment of the action of EGYT-475 (piberaline), BZP was found to potentiate the nerve-evoked release of NA.[14,15] Although BZP was found to inhibit the high-affinity uptake of DA and NA, it had a particularly significant blocking effect on 5-HT reuptake in rats.[5,15] It was also concluded that BZP had no effect on 5-HT_2 receptors and both inhibition of 5-HT uptake and 5-HT_1 receptor stimulation contributed to its central serotoninomimetic effect. Other researchers found BZP had 5-HT antagonistic and partial agonistic properties.[16] A study of dopamine-induced circling behaviour in rats indicated BZP caused the production of newly-synthesized DA.[17] Later rat studies found BZP caused the release of a dopamine transporter substrate (3(H)MPP+) *in vitro* and produced an *in vivo* increase in extracellular DA and 5-HT: the latter only at a high dosage – similar to methamphetamine.[17,18] TFMPP was found to be a selective releaser of 5-HT transporter substrate and increased extracellular 5-HT. Administration of BZP and TFMPP at a 3 mg/kg dose (1 : 1 ratio) produced parallel increases in 5-HT and DA, mirroring the results found with MDMA. A higher dose of 10 mg/kg BZP : TFMPP (1 : 1) increased DA to a higher degree than the drugs alone, with some rats developing seizures. This suggested a synergistic activity of BZP and TFMPP, mimicking the effects of MDMA at a molecular level, but with a lower potency.[18,19] Hashimoto *et al*. found that administration of BZP to rats reduced the levels of 5-HT and 5-hydroxyindole acetic acid in the cerebral cortex of animals that had previously been injected with MDMA, leading to the possibility that BZP might provide some protection against the neurotoxic effects of MDMA.[20]

Numerous *in vitro* and animal studies have been performed using mCPP. Overall, studies have demonstrated that mCPP affects all monoamine systems but particularly serotonergic pathways.[21–23]

Behavioural studies in animals

Rat studies have indicated that BZP produces potential amphetamine-like behaviour and heightened anxiety, the latter possibly due to interference in maturation of anxiety-associated forebrain mechanisms.[24] Rewarding properties were found to be mediated by the dopaminergic and serotonergic systems.[25] BZP has been shown to be a dose-dependent powerful locomotor stimulant.[18,19,26,27] These effects were not observed with TFMPP and, when administered in combination with TFMPP, only occurred at high doses (10 mg/kg BZP and TFMPP). As well as an increase in hyperactivity, it was also shown that repeated BZP exposure resulted in a sensitization and cross-sensitization to methamphetamine.[26] Mouse studies suggested that BZP has stimulant-like effects and that TFMPP has hallucinogen-like effects. However, MeOPP and mCPP did not appear to share these behavioral profiles.[27]

BZP and TFMPP were also studied in rhesus monkeys, whereby BZP substituted for cocaine in self-administration studies and amphetamine in discrimination studies, but the reinforcing effects of BZP : TFMPP were less than with BZP alone.[28] Signs of intoxication were observed following cocaine administration with BZP injected at doses of 0.1 and 0.3 mg/kg. Within the study, self-administered saline sessions suggested BZP had a fairly long-lasting behavioural effect. BZP also substituted for cocaine in rats and self-administration of BZP was acquired rapidly in drug-nadotlessive animals in a dopaminergic-mediated mechanism.[29]

Human studies

In a recent, randomized, double-blind, placebo-controlled study the subjective and physiological effects of BZP/TFMPP were investigated in 36 males. Participants were tested before and approximately 2 h after administration of a single dose of placebo or 100/30 mg BZP/TFMPP. The results revealed that BZP/TFMPP increases blood pressure and heart rate and subjective rating scales revealed that BZP/TFMPP has dexamphetamine-like effects, increases dysphoria and feelings of self-confidence.[30] In a related study involving 27 females, BZP at 200 mg was again found to increase blood pressure and heart rate and have stimulant effects, increased euphoria, dysphoria, sociability, and drug liking.[31]

Another recent randomized, double-blind, placebo-controlled study in New Zealand involved volunteers who had previously

used party pills containing BZP.[32] Participants received one of four treatments: (1) 300 mg/74 mg BZP/TFMPP and placebo, (2) 300 mg/74 mg BZP/TFMPP and 57.6 g alcohol, (3) placebo and 57.6 g alcohol, and (4) double placebo. Driving performance effects and physiological effects (adverse events, cardiovascular effects, psychological function, and delayed effects on sleep) were assessed. The study was stopped early, after 35 of the planned 64 subjects had undertaken testing, because of severe adverse events that occurred in 4 of 10 BZP/TFMPP-only subjects and 3 of 7 combined BZP/TFMPP and alcohol subjects. Severe events included agitation, anxiety, hallucinations, vomiting, insomnia, and migraine. Such events did not occur with the 6 placebo subjects and 12 alcohol-only subjects. BZP/TFMPP also resulted in increased heart rate and blood pressure and in difficulty in getting to sleep. BZP/TFMPP was found to significantly improve the driving performance, decreasing standard deviation of lateral position (SDLP) at −4.2 cm. SDLP, the degree to which individuals adjust lane position, has been found to be very sensitive to the influence of sedative medicinal drugs and alcohol. The effect of alcohol was to increase SDLP: 2.3 cm. Overall, it was concluded that BZP/TFMPP alone or with alcohol carries a significant risk of severe adverse events at the assumed doses studied but the study design has been criticized.[33]

In a previous study of former amphetamine addicts, the behavioural effects of BZP, d-amphetamine, and a lactose control were compared. The subjective effects of BZP and d-amphetamine were identical and liked by the volunteers.[13] There were changes in the excitation score, but no difference in the depression score after administration of the drugs. In an additional d-amphetamine comparative study in volunteers with no previous experience of amphetamines, both d-amphetamine and BZP produced a significant improvement in an auditory vigilance test.[11] No significant changes were found in tests of short duration (tapping rate, hand steadiness, and arithmetic), therefore the use of prolonged signal detection was recommended. Subjective effects of BZP (based on the volunteer selecting from a checklist of 41 adjectives) were only detected following a 100-mg dose (7.5 mg in the case of d-amphetamine). Overall, the studies concluded that BZP had a psychomotor stimulant response similar to d-amphetamine but d-amphetamine had an effective potency 10-fold greater than BZP. Of the physiological effects found in the studies, BZP (50 mg and 100 mg) was found to increase pulse rate, blood pressure (systolic and diastolic) and pupillary dilation.[12,13] These effects were comparable to those found with d-amphetamine but no change in pupil size was noted for d-amphetamine by Campbell *et al*.[13] Other tests observing the effect of BZP eye-drops on pupil diameter suggested an indirect sympathomimetic action.[12] During the study by Campbell *et al*., flushing and sweating were observed after BZP administration. Separately, Alansari and Hamilton (2006) reported that a 17-year-old male developed acute renal failure after consuming five BZP tablets and a small amount of alcohol.[34] A causal relationship with BZP toxicity was postulated in the absence of rhabdomyolosis.

For mCPP, a variety of studies have been performed involving humans, with a range of population types. mCPP is also the most extensively used probe of serotonin function in psychiatry.[35] In healthy subjects, oral and intravenous administration of mCPP have been shown to increase body temperature, blood pressure, and heart rate, and to cause anxiety, euphoria, nausea, dizziness, headaches, sweating, and reduced sleep. Overall, effects were dose-dependent and appeared greater with intravenous administration.[21] In a study of MDMA users where MDMA and mCPP were administered, mCPP did not affect heart rate and blood pressure (unlike administered MDMA). However, of the subjective effects, mCPP produced similar stimulant and hallucinogenic effects.[36]

Pharmacokinetics including metabolism

BZP and TFMPP

Studies with human liver microsomes found BZP and TFMPP are metabolized by the cytochrome P450 iso-enzymes; CYP2D6, CYP1A2 and CYP3A4.[37] This supports previous predictions based on animal studies. The drugs inhibited each other's metabolism, further indicating a potential issue with an interaction of the drugs if used in combination – as well as interactions with other drugs involving cytochrome P450. Animal and human studies have noted the same metabolites to be present for BZP: 4-hydroxy-BZP (4-OH-BZP or p-OH-BZP), 3-hydroxy-BZP (3-OH-BZP or m-OH-BZP), 4-hydroxy-3-methoxy-BZP, piperazine, benzylamine and N-benzylethylenediamine. The 4-hydroxy-BZP, 4-hydroxy-BZP and 4-hydroxy-methoxy-BZP metabolites are also excreted as glucuronide and/or sulfate conjugates in urine.[38–40] For TFMPP, animal and human studies have noted the same major metabolite to be present: 4-hydroxy-TFMPP (4-OH-TFMPP or p-OH-TFMPP) which is also excreted as glucuronide and/or sulfate conjugates in urine.[39–41] In rats receiving a single intraperitoneal 5 mg/kg dose of TFMPP, the cumulative amount of p-OH-TFMPP excreted within the first 48 h reached approximately 64% of the dose, of which 70% was the glucuronide conjugated form. The cumulative amount of parent TFMPP excreted was less than 0.7% of the dose.[40]

Human plasma concentrations of BZP were measured in blood samples taken from healthy adults (n = 7) over 24 h following a 200-mg oral dose of BZP. Concentrations were found to peak at 262 μg/l (C_{max}) and 75 min (T_{max}). Plasma concentrations of the major metabolites of BZP, 4-OH BZP and 3-OH BZP, were found to peak at 7 μg/l (at 60 min) and 13 μg/l (at 75 min), respectively. The elimination half-life ($t_{1/2}$) for BZP was found to be 5.5 hs. Clearance (Cl/F) was found to be 99 L/hour. The results of this study indicate that BZP may be detectable in plasma for up to 30 h following an oral dose. Additionally, 4-OH BZP, 3-OH-BZP and predominantly O-sulfate and N-sulfate BZP conjugate metabolites were also found in urine collected over the 24 h.[42]

Human plasma concentrations of TFMPP were measured in blood samples taken from healthy adults (n = 6) over 24 h following a 60-mg oral dose of TFMPP: these peaked at 24.10 μg/l (±1.8 μg/l) (C_{ma}) after 90 min (T_{max}). Plasma concentrations of 1-(3-trifluoromethyl-4-hydroxyphenyl)piperazine peaked at 20.2 μg/l (±4.6 μg/l) after 90 min. TFMPP had two disposition phases ($t(^1/_2)$ = 2.04 h (±0.19 h) and 5.95 h (±1.63 h). Apparent clearance (Cl/F) was 384 L/hour (±45 L/hr).[43]

MDBZP

In rats, studies have indicated that MDBZP is metabolized by demethylenation and subsequent methylation to N-(4-hydroxy-3-methoxybenzyl)piperazine followed by partial glucuronidation or sulfation.[44] Degradation of the piperazine moiety to N-(3,4-methylenedioxybenzyl)ethylenediamine and 3,4-methylenedioxybenzylamine and N-dealkylation to piperazine was also found.[44] Furthermore, MDBZP is a primary metabolite of fipexide.[45]

MeOPP

In Wistar rats, pMeOPP was found to be metabolized *in vivo* mainly by O-demethylation to 1-(4-hydroxyphenyl)piperazine (4-HO-PP) in addition to degradation of the piperazine moiety.[46] Staack *et al*. also studied the O-demethylation process with cDNA-expressed human hepatic cytochrome P450 enzymes in pooled human liver microsomes and in single donor human liver microsomes with CYP2D6 poor metabolizer genotype. It was found that the CYP2D6 isoenzyme catalyzed O-demethylation. The CYP2D6-specific chemical inhibitor quinidine significantly inhibited 4-HO-PP formation, in incubation mixtures with pooled microsomes and pMeOPP. Furthermore, O-demethylation was found to be significantly lower in poor metabolizing genotype microsomes compared with pooled microsomes (70.6% +/− 7.2%). This suggested the involvement of polymorphic CYP2D6 as the enzyme mainly responsible for pMeOPP O-demethylation.[46] In rat urine, 4-hydroxyaniline was detected in addition to 4-HO-PP.[47] The presence of these compounds may suggest pMeOPP use; however, additional metabolites could be less specific and may be related to oMeOPP use. Furthermore, oMeOPP is a metabolite of some prescribed drugs: enciprazione, milipertine, urapidil, dropropizine and oxypertine.[1,47]

mCPP

mCPP is a metabolite of the anti-depressant drugs trazodone, nefazodone, enziprazole and etoperidone and of the minor tranquillizer mepiprazole.[1,48–50] This has been found in both animal and human studies with mCPP formed by dealkylation via CYP3A4.[51] A number of human studies have been performed involving administration of mCPP directly. The major metabolite of mCPP was found to be p-OH-mCPP (para-hydroxy-mCPP) via CYP2D6-mediated hydroxylation.[39,49] Due to the involvement of cytochrome P450 in the metabolism of precursor drugs and mCPP itself, the co-ingestion of these drugs as well as genetic polymorphisms and drug inducers or inhibitors could affect the metabolism and plasma concentration of mCPP and its metabolites. For example, co-administration of fluoxetine was found to produce a 4-fold increase in mCPP plasma concentrations.[51] In humans, it was found that maximum mCPP concentrations varied 2.3-fold after intravenous infusion and 8-fold after oral administration.[53] The absolute bioavailability ranged from 12% to 84% with an elimination half-life ranging from 2.4 h to 6.8 h after intravenous infusion and from 2.6 h to 6.1 h after oral administration. However, the authors stated that the kinetic data as well as the pharmacodynamic response varied to an extent that precluded pharmacokinetic-pharmacodynamic modelling. The wide inter-individual variability in its disposition kinetics could not be fully explained by genetic variation of the mCPP-metabolizing CYP2D6 enzyme.[53] In a previous study, 8 female and 6 male healthy volunteers were included in a randomized, double-blind, double-dummy, three-way crossover design of single-dose intravenous (0.1 mg/kg), oral (0.5 mg/kg), and placebo treatment, with 24-h follow-up. mCPP showed a large variability in clearance (11–92 ml/hr) and bioavailability (14–108%).[54]

Analytical toxicology

Extraction and analysis of the piperazines is relatively straightforward given their chemically basic nature and structure makes them amenable to a number of techniques. Detection methods such as capillary electrophoresis (CE), gas chromatography with mass-spectrometry (GC-MS), high performance liquid chromatography with diode-array detection (HPLC-DAD) and/or mass-spectrometry (LC-MS), infra-red spectroscopy (IR) and thin layer chromatography (TLC) have been published.[1,3,44,47,55–59] Of course, the detection outputs depend on the technique used but as an example, for BZP, the underivatized GC-electron impact mass spectrum has ion peaks at (m/z) = 91 (base peak); 134, 56, 176 and 65. Similarly, with LC-MS, the pronated molecular ion [M+H] of 177 m/z is observed with fragmentation resulting in the predominant 91 m/z ion. For presumptive tests, BZP does not give a colouration with Marquis or Scott's field tests, but does give a positive reaction with Nitroprusside reagent. There is also some cross-reactivity with commercially available urine immunoassay tests for methamphetamine.[58]

As indicated above, although various techniques have been reported, primarily GC-MS and LC-MS have been used for the identification and quantification of piperazines in body fluids.[1,38,40,43,60–63] Furthermore, HPLC-DAD has been shown to allow identification of positional isomers of piperazine derivatives (e.g. 3-TFMPP and 4-TFMPP) which is not possible with GC-MS or LC-MS in the absence of chromatographic separation due to similarity in fragmentation ions.[3]

Toxicology

Overall, BZP appears to produce stimulant and toxic effects similar to amphetamines and other sympathomimetics. TFMPP is commonly used in conjunction with BZP in order to seek the entactogenic effects of MDMA. Adverse effects may occur when BZP is co-ingested with other drugs (in particular MDMA and other serotonergic/dopaminergic compounds), but toxic effects with BZP alone have also been reported. Agitation, tachycardia, and seizures have been noted. User reports and study information indicates that TFMPP appears to produce mild stimulant and hallucinogenic effects but toxic effects with TFMPP alone have not been reported.

For mCPP, user reports and study information indicates that it produces stimulant, anxiety and hallucinogenic effects. Additional adverse effects include nausea, headache, dizziness, sweating, and potential cardiovascular symptoms (e.g. increased heart rate and blood pressure); although the latter effects are not exhibited in some studies.[36] Despite the relatively high number of human studies involving mCPP administration, there are a very limited number of cases[64] and user reports even on the Internet (Erowid and The Lycaeum). The human study data invariably involve mCPP alone, whilst the limited user reports generally mention the use of other drugs of abuse. This introduces the possibility of drug-drug interactions or exacerbation of toxic effects for poly-drug users.

For MDBZP, there are no specific data; however, of indirect relevance, fipexide use has been reported to result in fever and serious liver cell necrosis and fulminant liver failure.[65] There are no study data to suggest or confirm that MDBZP (as an active metabolite) was the cause of these effects.

pMeOPP user reports indicate it produces mild stimulant and some hallucinogenic effects. Additional adverse effects include nausea but no human studies have been performed and there are a very limited number of user reports, even on the Internet (Erowid and The Lycaeum). Although some user reports involve pMeOPP alone, a few others also mention the use of other drugs of abuse. This again introduces the possibility of drug-drug interactions or exacerbation of toxic effects for poly-drug users.

In the mid-2000s, a clinical study and survey in New Zealand involved individuals purported to have used 'party pills'.[7,8] Other drugs of abuse and alcohol were also believed to have been taken in some cases but the toxicological status and identity of any piperazines was not supported by analysis. Symptoms noted were anxiety, vomiting, headache, agitation, palpitations, confusion, collapse and seizures; some symptoms had persisted for 24 h post-ingestion. Other features included tachycardia and hypertension with a prolonged QTc in some clinical patients. Physical problems reported in a survey were (in order of frequency): poor appetite, hot/cold flushes, heavy sweating, stomach pains/nausea, headaches and tremors/shakes. Psychological problems experienced were (in order): trouble sleeping, loss of energy, strange thoughts, mood swings, confusion, and irritability.

Cases of piperazine derivative intoxication in humans

There have been various reports of non-fatal and fatal intoxication where piperazines have been found. However, a major problem in investigating the involvement of piperazines in hospital admissions and fatalities is the potential lack of laboratory confirmation or diagnosis. Although numerous methods have been published, piperazines are not always included in routine or targeted toxicological analysis. Alternatively they may be detected but not identified as being piperazines. This was partly due to the lack of reference materials for some of the piperazine derivatives but some of the more commonly abused substances are now available for analysis.

Non-fatal cases

Details of three patients in New Zealand were reported by Gee *et al*.[7]

Patient 1: (16-year-old female, 4 pills, no alcohol) had a tonic clonic seizure 2.5 h after her last tablet. Additional seizures were treated with diazepam. GCS 3/15 with intubation. Heart rate (HR) 149 bpm, BP 70/55, blood glucose 5.6 mmol/L, temperature 36 °C. After further seizures she had a metabolic and respiratory acidosis. She was transferred to ITU but extubation was possible 12 h later (GCS 15/15). Laboratory analysis showed BZP and metabolites only. No apparent prolonged adverse effects were reported a week later.

Patient 2: (18-year-old female) had five seizures with metabolic and respiratory acidosis. Transferred to ITU but later extubated with no apparent long-term effects. Laboratory analysis showed BZP only.

Patient 3: (25-year-old male, 2 pills with alcohol and 2 pills following morning) had a tonic seizure 3 h after last tablet whilst driving a car. HR 170 bpm, BP 148/75, blood glucose 5.4 mmol/L. Drowsy but conversant upon admission. Laboratory analysis showed BZP metabolites and alcohol only.

Comprehensive case details of two further cases of BZP intoxication have also been published.[66] Both cases required prolonged hospital care but survived. A 19-year-old female developed status epilepticus, hyperthermia, disseminated intravascular coagulation, rhabdomyolysis, and renal failure associated with BZP ingestion whilst in police custody. She was transferred to hospital and a plasma BZP concentration of 0.20 mg/L was measured 10 h after being in custody. Benztropine metabolites (patient's medication), nicotine and caffeine were additionally detected in the urine. Case 2 involved a 22-year-old male who developed a similar toxicity from the combined use of BZP and MDMA. Concentrations measured in a blood sample collected 3 h after admission found a BZP concentration of 2.23 mg/L and an MDMA concentration of 1.05 mg/L. A repeat BZP blood level 6 h later was 0.1 mg/L.

Gee *et al*. also reported plasma BZP concentrations in 96 individuals measured on admission to an emergency department over a 2-year period following use of 'party pills'.[67] The concentrations were between 0 and 6.29 mg/L (mean 0.68 mg/L). Patients with concentrations between 0.0 and 0.50 mg/L tended to report symptoms of anxiety, palpitations, and vomiting. Agitation, anxiety, and confusion were more frequent above 0.5 mg/L. Seizures were associated with levels as low as 0.05 mg/L but increased with higher concentrations and were consistent when plasma levels were above 2.15 mg/L. This took into account the entire cohort of 96 patients who had plasma BZP concentrations measured, whether or not they had co-ingested ethanol.

In May 2006, 7 patients (18–23 years) attended an Accident and Emergency Department in London, UK, from the same nightclub having ingested supposed 'Ecstasy' or amphetamine tablets (4–9 tablets consumed).[63,68] The diamond-shaped tablet ingested by the individuals was found to contain only BZP. Two of the individuals collapsed in the club with witnessed self-terminating grand mal seizures. Upon admission, five of the patients exhibited dilated pupils, anxiety, agitation and tachycardia. After 8 h of observation and treatment with benzodiazepines, there was no evidence of continued toxicity. Serum samples were analyzed in four of the patients and revealed BZP concentrations of 1.3, 1.9, 1.9, and 2.5 mg/L.[63] No other piperazines, drugs, or alcohol were detected. Clinical information was published for one of the female patients, detailing a seizure in the club, and was agitated, tachycardic (156 bpm), BP 150/51, apyrexial (temperature 35.9 °C) and had dilated pupils and a GCS of 15/15. She was discharged after 12 h.[67]

The same authors also published instances of three patients presenting to the emergency department after ingesting four tablets thought to be Ecstasy over the course of an evening.[69] It was reported that they presented with dissociative-type symptoms, nausea, and signs consistent with sympathomimetic toxicity. All three improved with conservative management and observation, within 12 h of presentation. TFMPP and BZP were found in serum at concentrations of 263 +/− 5.8 μg/L (range 260–270 μg/l) and 46.7 +/− 15.3 μg/l (range 30–60 μg/l), respectively. No other recreational drugs were detected in the blood and urine samples.

Elsewhere in the UK, between June 2006 and November 2007, BZP was detected in 11 patients, with TFMPP detected in 9 of the cases by this author. All cases were confirmed by toxicological analysis. Urine concentrations ranged between 5.21 and 202.7 mg/l (BZP) and 0.40–20.66 mg/l (3-TFMPP). Blood samples were only available in three cases. Case 1 (25-year-old male) serum BZP = 0.17 mg/l. MDMA and citalopram were also present. The patient presented with hyponatraemia, mydriasis and prolonged respiratory depression. Case 2 (24-year-old male) plasma BZP = 0.32 mg/l, 3-TFMPP = 0.08 mg/l. Two BZP tablets were reported to have been taken. Insufficient sample volume was available for additional drug analysis. Case 3 (age/sex not known) plasma BZP = 0.47 mg/l, 3-TFMPP = 0.10 mg/l. No other drug or clinical information was available.

Fatal cases

There have been very few instances of fatalities involving piperazines reported in the literature. Six cases have been formally published and primarily involve BZP with 3-TFMPP in two cases.[3,6,70] None involved piperazines alone.

Table 1. Compilation of post-mortem data for fatalities where piperazines have been detected

Case No.	Other cause?	Deceased age/sex	PM Urine piperazines	PM Blood piperazines	Other drugs detected
1	Hanging	34-year-old male	Not available	BZP 0.68 mg/l 3-TFMPP 0.04 mg/l	Not available
2	Hanging	23-year-old male	BZP 3-TFMPP	BZP 3.20 mg/l	Cocaine MDMA Levamisole Cannabis Alcohol
3	Pedestrian RTA	33-year-old male	BZP 3-TFMPP	BZP <0.3 mg/l 3-TFMPP <0.03 mg/l	Methadone Alcohol
4	Heroin	30-year-old male	Not available	BZP 0.68 mg/l 3-TFMPP 0.04 mg/l	Morphine Benzodiazepines
5	None	19-year-old male	BZP 3-TFMPP	BZP 0.69 mg/l 3-TFMPP 0.08 mg/l	Lignocaine
6	None	26-year-old male	BZP 3-TFMPP	BZP 1.91 mg/l 3-TFMPP 0.07 mg/l	Cannabis Cocaine Levamisole Lignocaine Diazepam Alcohol
7	Methadone	35-year-old male	BZP 3-TFMPP	None detected	Methadone Diazepam
8	Heroin	25-year-old male	BZP 3-TFMPP	None detected	Morphine Cannabis Cocaine Trimethoprim Alcohol
9	None	36-year-old male	BZP 3-TFMPP	BZP <0.3 mg/l	Lansoprazole Amphetamine
10	RTA	22-year-old male	BZP 3-TFMPP	BZP 1.44 mg/l 3-TFMPP 0.20 mg/l	Alcohol Cannabis
11	Hanging	33-year-old male	BZP 3-TFMPP	BZP 1.12 mg/l 3-TFMPP 0.30 mg/l	Ketamine Alcohol
12	None	38-year-old male	BZP 3-TFMPP	None detected	Cannabis Venlafaxine Paracetamol
13	Heroin	45-year-old male	BZP 3-TFMPP	None detected	Cannabis Diazepam Olanzapine Amitriptyline Morphine
14	None	38-year-old male	BZP 3-TFMPP	None detected	Paracetamol Promethazine Venlafaxine
15	Heroin	33-year-old male	BZP 3-TFMPP	3-TFMPP <0.03 mg/l	Cocaine Morphine Levamisole Alcohol
16	Hanging	25-year-old male	BZP 3-TFMPP	None detected	Cannabis Methadone Diazepam Citalopram Alcohol
17	Heroin	27-year-old male	BZP 3-TFMPP	None detected	Morphine

Table 1. (*Continued*)

Case No.	Other cause?	Deceased age/sex	PM Urine piperazines	PM Blood piperazines	Other drugs detected
18	None	23-year-old male	BZP 3-TFMPP	None detected	Amitriptyline Diazepam Methadone Cannabis Ketamine Morphine Sildenafil
19	None	48-year-old male	BZP 3-TFMPP	3-TFMPP <0.03 mg/l	Alcohol

In Sweden, Wikström *et al*. reported the presence of BZP in postmortem blood at a concentration of 1.7 mg/l, in addition to MDMA, MDA and tetrahydrocannabinol (THC).[6] A further fatality was mentioned also with a BZP blood concentration of 1.7 mg/l.[6] Amphetamine, MDMA and THC were detected as well. No further details regarding the circumstances of these deaths were described.

Balmelli *et al*. published a fatality involving a 23-year-old female in Switzerland.[70] She was admitted to hospital with headache, malaise and somnolence 11 h after ingestion of BZP and 7 h after ingestion of MDMA along with large volumes of fluids. She also presented with bradycardia (48 bpm), hypertension (BP 154/95), hypnonatraemia (sodium 115 mmol/l) and a GSC of 6. She suffered two fits and required intubation. A CT scan indicated a cerebral oedema and, although the sodium levels returned to normal within 38 h post-admission, she deteriorated neurologically with increasing tonsillar herniation and died 57 h after initial presentation. In this case, the hypnonatraemia was associated with the intake of fluids after MDMA ingestion, and therefore the specific contribution of BZP was difficult to determine.

This author has previously presented three fatalities in the UK.[3] Case 1: A 26-year-old male driver was involved in a fatal road traffic accident. Subsequent information indicated he may have used 'Wicked High' pills. Comprehensive toxicological analysis of postmortem blood and urine samples found a urinary BZP of 15.73 mg/l, 3-TFMPP of 1.04 mg/l, as well as cannabis, cocaine, ephedrine, MDMA, ketamine and ethanol (128 mg/dl). The blood concentrations were; BZP (0.71 mg/l), 3-TFMPP (0.05 mg/l), ketamine (0.96 mg/l) and ethanol (77 mg/dl).

Case 2: A 32-year-old male was the driver of vehicle that struck a tree. He was taken to hospital but later died. Comprehensive toxicological analysis of postmortem blood and urine samples found a urinary BZP of 4.88 mg/l, cannabis, benzodiazepines, cocaine, diltiazem, amphetamine, MDMA, and ketamine. No alcohol was detected. Blood analysis showed BZP (<0.50 mg/l), ketamine, MDMA (0.54 mg/l), amphetamine, diazepam, cocaine, cyclizine, and atracurium. No alcohol was found. There was insufficient sample volume for measurement of the additional drugs present.

Case 3: A 17-year-old male fell through the roof of a building having walked across it whilst taking a shortcut. He had been to a party and may have taken Ecstasy and drank alcohol. Comprehensive toxicological analysis of postmortem blood and urine samples found a urinary BZP of 8.72 mg/l, 3-TFMPP of 0.92 mg/l and ethanol (248 mg/dl). The blood analysis showed BZP (1.39 mg/l), 3-TFMPP (0.15 mg/l) and ethanol (140 mg/dl).

Subsequently, BZP and 3-TFMPP have been found in a further 19 fatalities between 2007 and 2010 and are summarized in Table 1. Both drugs were detected together in each case, suggesting possible concomitant ingestion. No other piperazines have been found in fatalities investigated by this author. Of the cases, six involved a mechanical cause of death (e.g. RTA, hanging), six cases were likely due to other drug use (e.g. heroin, methadone) and seven cases had no obvious alternative cause of death. However, due to the presence of other drugs, medical history, and case circumstances, the actual significance of BZP or 3-TFMPP was unclear. In fact the highest post mortem blood concentration (3.20 mg/l BZP) was found where the manner of death was hanging.

Conclusions

Although many piperazine derivatives exist, only a limited number have been studied, whereby they have been found to be generally stimulant in nature resulting from dopaminergic, noradrenergic, and predominantly serotoninergic effects in the brain. Reported toxic effects include agitation, anxiety, cardiac symptoms (e.g. tachycardia) and sometimes seizures. As for many drugs they are primarily metabolized by cytochrome P450 with subsequent possible glucuronidation and/or sulfation. BZP and TFMPP have been reported to inhibit each other's metabolism, further indicating a potential issue with an interaction of the drugs if used in combination – as well as interactions with other drugs involving cytochrome P450. Their abuse has only been relatively recently observed in the last decade with only a few identified in biological fluid (primarily BZP and 3-TFMPP) despite publications of a number of analytical methods. However, even when detected, the toxicological significance of their presence is often difficult to ascertain as many cases involve other drugs as well as a wide and overlapping range of concentrations found in blood (both in life and after death).

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