

## Piperazines – The New Emerging Recreational Drugs

by Jennifer Button and Susannah Kenyon on behalf of the Young Scientists Interest Group

The market for ‘legal highs’ or ‘party pills’ has expanded enormously in the UK over the last two years. The ‘new’ and popular compound found in tablets and capsules sold in online and herbal shops, is a group of drugs called piperazines. Benzylpiperazine (BZP) is the most common derivative, often found in conjunction with trifluoromethylphenylpiperazine (TFMPP). Other piperazine benzyl/phenyl derivatives seen in tablets and capsules include; 1-(4-fluorophenyl)piperazine (pFPP), 1-(3-chlorophenyl)piperazine (mCPP), 1-(4-methoxyphenyl)piperazine (pMeOPP) and 1,4-dibenzylpiperazine (DBP). These are being actively marketed under names such as A2, Rapture, Jump, Frenzy, Nemesis, Euphoria, ESP and Pep.

Despite being entirely synthetic, piperazines are frequently marketed as ‘natural’ or ‘herbal’ products, due to their erroneous association with the pepper (*Piper*) plant. Piperazines are sold as ‘herbal ecstasy’ and are being promoted as legal alternatives to illicit drugs such as 3,4-methylenedioxymethamphetamine (MDMA).<sup>1</sup> They are even being described as “drug harm minimisation solutions”<sup>2</sup> and their use referred to as “positive risk taking,” as the risks posed by these are conceived to be less than those of other illicit drugs.<sup>3</sup>

Although not herbal, the popularity of piperazines is still growing due to their legal status in some jurisdictions, weak psychoactive effects and perceived safety compared to illicit drugs. The general public naively think that because these products are legal and being sold openly, they must be safe. The truth is that the ‘legal high’ industry is unregulated and new piperazine derivatives remain untested and not fully evaluated for safety. Piperazines have been popular in New Zealand (NZ) for many years, and their popularity has now spread to the UK. In a report on ‘BZP Industry Advice to the Advisory Council for the Misuse of Drugs (ACMD)’, Spiritual High Ltd., one of four major distributors, claims to have been shipping up to 3,600 doses of BZP a day into the UK.<sup>3</sup>

Piperazine is not a new compound. It was first synthesised in 1944 by the Wellcome Research Laboratories as a potential anti-helminthic agent for livestock.<sup>4</sup> Its use for this purpose declined due to poor efficacy and adverse effects such as seizures.<sup>4</sup> In human trials benzylpiperazine derivatives resulted in involuntary head movements, reduced reaction times, hyperactivity and agitation.<sup>5</sup> Studies in the 1970s and 1980s found BZP to possess anti-depressant activity and stimulant properties with abuse potential.<sup>5-8</sup> These studies suggested that BZP has approximately one-tenth of the potency of amphetamine. A double blind study in 1973 even found that former amphetamine addicts were unable to distinguish between equipotent doses of BZP and amphetamine.<sup>7</sup>

Studies in rats have shown that BZP elevates serotonin and dopamine neurotransmitters in the brain by blocking the reuptake of these transmitters at the synapse.<sup>9</sup> TFMPP was also found to be a selective releaser of serotonin in the rat brain.<sup>9</sup> This can be compared to MDMA that acts as a substrate for dopamine and serotonin transporters in brain tissue, elevating synaptic serotonin and dopamine levels.<sup>9-11</sup> However, it has been shown that BZP and TFMPP are less potent individually than MDMA.<sup>9</sup> This is mirrored by comments from ecstasy users that BZP does not give such euphoric effects as MDMA, but does result in greater insomnia and hangover.<sup>12</sup> Rat

studies in which BZP and TFMPP were used together showed an increase in the release of dopamine that is greater than the summed effect of either drug alone.<sup>9,11</sup>

Whilst the metabolism of piperazines has been extensively studied using animal models<sup>13-19</sup> and *in-vitro* models,<sup>17</sup> to date little information is available about their *in-vivo* metabolism in humans. Staack *et al.* (2002) concluded that BZP is metabolised to a considerable extent, after the identification of 3'-hydroxy-BZP, 4'-hydroxy-BZP, 4'-hydroxy-3'-methoxy-BZP, piperazine, benzylamine and N-benzylethylenediamine in rat and human urine by gas chromatography-mass spectrometry (GC-MS).<sup>13</sup> They also postulated the formation of benzoic acid as a metabolite of BZP. From these metabolites they proposed that the cytochrome P450 enzyme system, CYP2D6 and catechol-*O*-methyl-transferase (COMT) were key components in the breakdown of BZP.<sup>13</sup> The same authors later (2003) studied the metabolism of pMeOPP identifying, amongst other compounds, 1-(4-hydroxy phenyl)piperazine and 4-hydroxyaniline (4-HO-PP) as major metabolites.<sup>14</sup> Staack *et al.* (2005) have recently reviewed the metabolism of designer drugs of abuse, including the piperazine family of drugs.<sup>20</sup>

Some of the metabolites of BZP, MeOPP and potentially other piperazines, are common to several compounds. The BZP metabolite, 4'-hydroxy-3'-methoxy-BZP, is also a metabolite of another piperazine, 1-(3,4-methylenedioxy-benzyl)piperazine (MDBP).<sup>13</sup> Whereas MeOPP shares its major metabolite with the antitussive dropropizine, the antipsychotics oxypertine and fluanisone and the antihypertensive drug urapidil,<sup>13,14</sup> the acetylated MeOPP metabolite, 4-hydroxyaniline is also identical to acetylated paracetamol.<sup>14</sup> In addition, the piperazines themselves are also produced as metabolites of other medicaments such as piberaline (BZP), antrafenine (TFMPP), nefazadone, trazadone, etoperidone, entiprazole and mepiprazole (mCPP) and enciprazione, milipertine and urapidil (oMeOPP).<sup>21</sup> These are important considerations when interpreting toxicological findings.

Piperazines can cause unpredictable and serious toxicity and the true health consequences of their widespread availability are beginning to emerge with numerous reports of hospitalisations and even fatalities.<sup>4,22-26</sup> Although, piperazines are commonly taken in combination with other illicit drugs, such as amphetamine and MDMA, and the involvement of these, in cases of toxicity or fatality, cannot always be ruled out.<sup>22-23</sup> The first reported case of BZP toxicity in the UK, presented in May 2006.<sup>26</sup> Reported side effects have included insomnia, anxiety, nausea, vomiting, hyperthermia, cardiac arrhythmias, tachycardia, abdominal pain, acute renal failure and acute psychosis. Seizures are a common presentation amongst reported cases of piperazine toxicity. On their website Spiritual High Ltd. describe how BZP long term effects could put a stress on the heart due to increased blood pressure.<sup>2</sup> Legal High tablet and capsule packaging states that alcohol should not be taken in conjunction with BZP.<sup>2</sup> Despite this warning many still combine the two. In an analytical cross-sectional study of 1043 people attending an Emergency Department in Hamilton, NZ, 66.4 % of those confessing to having used the so called 'herbal party pills' had also consumed alcohol at the same time.<sup>27</sup>

Typical doses range from 60 to 200 mg of BZP, but some capsules claim to contain 1000 mg and no ranges are given for other derivatives.<sup>2,3</sup> Case studies reporting the adverse effects of piperazines suggest that it is not uncommon for an average of four to five tablets to be consumed during a night out.<sup>4,24-26</sup> Products are priced at between GBP5 and GBP10 (US\$10-20) for a pack containing two to three capsules.<sup>3</sup> In an unpublished study by Kenyon *et al.* (2006) 35 different piperazine tablet and capsule varieties were purchased from three different Internet suppliers, and were analysed by GC-MS to determine the identity and quantity of piperazines present. Additional and

different piperazine derivatives were often found and not declared on the packaging. The quantities of the major ingredients, BZP and TFMPP, were also found to be highly variable and unpredictable (further details to be presented at 2007 IATDMCT Congress in Nice, France). In contrast de Boer *et al.* (2001) analysed the contents of a single 'A2' capsule by gas chromatography-nitrogen phosphorous detection (GC-NPD) and measured 86.4 mg of BZP: this showed good correlation with the packaging.<sup>21</sup>

The USA Food and Drug Administration (FDA) is concerned about the public health implications of Internet drug sales.<sup>28</sup> Currently the legal highs industry sits in a grey area of the law between medicines and food, protected by the anonymity of the Internet. Recently the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) released a report on BZP as a new psychoactive substance and concluded that further investigation into the health and social risks of BZP was necessary.<sup>29</sup> UK and NZ drug regulatory authorities are currently reviewing the legal status of the piperazines. Although not controlled by the Misuse of Drugs Act, the Medicines and Healthcare products Regulatory Agency (MHRA) published a press release recently warning that BZP is dangerous and illegal to sell under the UK Medicines Act.

BZP is currently legal to sell in NZ under herbal and dietary supplement legislation. A new class D was added to its drug categorisation in 2005, restricting the sale of BZP to those over 18 years of age.<sup>30</sup> BZP became regulated in Sweden and Japan under the Prohibition of Certain Goods Dangerous to Health Act 2003 and Narcotics and Psychotropics Control Law 2003, respectively.<sup>31</sup> In 2004 the USA Drug Enforcement Administration (DEA) placed BZP into schedule 1 of the Controlled Substance Act, making it illegal in the US, although TFMPP remains unscheduled.<sup>31</sup> BZP is controlled in Australia and parts of Europe.

Many commercially available urine screening kits for drugs of abuse have only low cross reactivity with piperazines, if any at all, and are not suitable for their detection. De Boer *et al.* (2001) did not detect BZP in urine spiked with 100,000 ng/mL using the Abbott AxSYM<sup>®</sup> amphetamine / metamphetamine II FPIA.<sup>21</sup> However, the Dade Behring EMIT<sup>®</sup> d.a.u<sup>®</sup> amphetamine kit did show some cross reactivity<sup>21</sup> and Staack *et al.* (2002) reported cross reactivity with the Abbott amphetamine / metamphetamine II FPIA assay on the TDx platform at BZP concentrations exceeding 100,000 ng/mL.<sup>13</sup> BZP and TFMPP-HCL tested positive using the CEDIA<sup>®</sup> DAU amphetamine / ecstasy assay at the 1000 ng/mL cut off at tested concentrations of 150,000 and 25,000 ng/mL respectively.<sup>32</sup>

GC-MS with and without derivatisation has, so far, been the method of choice for analysis of piperazines. In many presented methodologies, piperazines were incorporated into systematic toxicological analysis and were analysed using full scan GC-MS<sup>13-16,19</sup>, although other authors have presented methods based on single ion monitoring (SIM).<sup>21</sup> Derivatisation was achieved by acetylation, trifluoroacetylation, heptofluorobutyrylation or methylation, after liquid-liquid extraction.<sup>13-16,19,21,31</sup> Tsutsumi *et al.* (2005) employed solid phase extraction technology (OASIS HLB/MCX) prior to liquid chromatography-electrospray ionisation mass spectrometry (LC-ESI-MS) analysis.<sup>31</sup> GC-NPD and high performance liquid chromatography-ultra violet detection (HPLC-UV, BZP  $\lambda_{\max}$ =211nm) have also be utilised in the detection of piperazines.<sup>21</sup> Methods have focused on the analysis of blood and urine (after acid hydrolysis). Typical blood concentrations of BZP detected in drug abusers have ranged from 0.02 to 2.5 mg/L with 1.7 mg/L measured in one autopsy case.<sup>23,26</sup>

In conclusion the pharmacodynamic effects of piperazines may be their limiting factor; the prolonged insomnia and strong hangover effects are more pronounced than those produced by MDMA and amphetamine. Although they do serve their purpose as a

mild hallucinogen, the market may simply lose interest in a group of drugs that are not actually as effective as those that are illegal. If they are found to be detrimental to health in the short and long term, they may become controlled. This, as Spiritual High Ltd. suggests, may even increase their appeal to drug users with the ‘forbidden fruit effect’.<sup>3</sup>

Monographs detailing the analytical properties of the piperazines are presented on the London Toxicology Group website; [www.ltg.uk.net](http://www.ltg.uk.net)

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### References

- 1) EMCDDA Annual report: [www.emcdda.europa.eu](http://www.emcdda.europa.eu)
- 2) [www.spiritualhigh.co.uk](http://www.spiritualhigh.co.uk) accessed 09 Nov 2006
- 3) Spiritual High Ltd. BZP Industry Advice to the ACMD. (2006)
- 4) Gee et al. *New Z Med J.* (2005) 118:1227
- 5) Bye et al. *Eur J Clin Pharmacol.* (1973) 6(3):163
- 6) Tekes et al. *Pol. J. Pharmacol Pharm.* (1987) 39:203
- 7) Campbell et al. *Eur J Clin Pharmacol.* (1973) 6(3):170
- 8) Magyar. *Pol. J. Pharmacol Pharm.* (1987) 39:107
- 9) Baumann et al. *Neuropsychopharm.* (2005) 30:550.
- 10) Green et al. *Pharmacol Rev* (2003) 55:463
- 11) Baumann et al. *Ann N Y Acad Sci* (2004) 1025:189.
- 12) [www.erowid.org](http://www.erowid.org) accessed 09 Nov 2006
- 13) Staack et al. *J Chromatogr B* (2002) 773(1):35
- 14) Staack et al. *J Chromatogr B* (2003) 798:333
- 15) Staack et al. *J Mass Spec* (2003) 38:971
- 16) Staack et al. *J Anal Toxicol.* (2003) 27:560
- 17) Staack et al. *Biochem Pharm* (2004) 67:235
- 18) Staack et al. *Xenobiotica* (2004) 34(2):179
- 19) Staack et al. *J Mass Spec* (2004) 39:255
- 20) Staack et al. *Curr Drug Metab* (2005) 6:259
- 21) De Boer et al. *Forensic Sci Int.* (2001) 47
- 22) Balmelli et al. *Dtsch Med Wochenschr.* (2001) 13;126(28-29):809
- 23) Wilkstrom et al. *J Anal Toxicol.* (2004) 28:67
- 24) Austin et al. *Aust Psych.* (2004) 12(4):406.
- 25) Alansari et al. *NZMJ.* (2006) 119(1233):U1959
- 26) Wood et al. *Lancet.* (2007) 369:1490
- 27) Nicholson. *Emerg Med Australasia* (2006) 18:180
- 28) Henney. *J Med Internet Res* (2001).3(1):e3
- 29) Europol-EMCDDA Joint Report on a new psychoactive substance: 1-benzylpiperazine (BZP)
- 30) Transform Drug Policy Foundation Briefing (Oct 2006)
- 31) Tsutsumi et al. *J Chromatogr B* (2005) 819(2):315
- 32) Microgenics CEDIA<sup>®</sup> Amfetamine / Ecstasy Cross Reactivity Table

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**Figure 1.** Examples of tablets and capsules, with their packaging, available on the Internet (a) from left to right, ‘Wicked Highs Coffee’ from [Wicked Highs](http://www.wickedhighs.co.uk), ‘Extreme Energy Pills’, ‘Jump Energy Burn’ and ‘Jax Extra Strength’ by London Underground, all available from [www.everybodydoesit.co.uk](http://www.everybodydoesit.co.uk). (b) from left to right, P.E.P X, P.E.P Love, P.E.P Stoned, P.E.P Twisted and P.E.P Pills, all available from [Spiritual Highs.co.uk](http://SpiritualHighs.co.uk).