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Review

The risk of chronic psychedelic and MDMA microdosing for valvular heart disease

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Abstract

Psychedelic microdosing is the practice of taking very low doses of psychedelic substances, typically over a longer period of time. The long-term safety of chronic microdosing is relatively uncharacterized, but valvular heart disease (VHD) has been proposed as a potential risk due to activation of the serotonin 5-HT_{2B} receptor. However, this risk has not yet been comprehensively assessed. This analysis searched for all relevant in vitro, animal, and clinical studies related to the VHD risk of lysergic acid diethylamide (LSD), psilocybin, mescaline, N,N-dimethyltryptamine (DMT), and the non-psychedelic 3,4-methylenedioxymethamphetamine (MDMA). All five compounds and some metabolites could bind to the 5-HT_{2B} receptor with potency equal to or greater than that of the 5-HT_{2A} receptor, the primary target of psychedelics. All compounds were partial agonists at the 5-HT_{2B} receptor with the exception of mescaline, which could not be adequately assessed due to low potency. Safety margins relative to the maximum plasma concentrations from typical microdoses were greater than known valvulopathogens, but not without potential risk. No animal or clinical studies appropriately designed to evaluate VHD risk were found for the four psychedelics. However, there is some clinical evidence that chronic ingestion of full doses of MDMA is associated with VHD. We conclude that VHD is a potential risk with chronic psychedelic microdosing, but further studies are necessary to better define this risk.

Keywords

Psychedelics, microdosing, valvular heart disease

Introduction

Psychedelics are a class of drugs that can produce profound changes in perception, cognition, and emotion in moderate to high doses. The low-dose use of psychedelics, colloquially known as "microdosing," has increased in recent years in both popular practice and academic attention paid to it (Polito and Liknaitzky, 2022). Microdosing involves sub-hallucinogenic doses that are not overtly intoxicating, often taken chronically over a longer period of time (Kuypers et al., 2019; Liechti and Holze, 2022). Surveys indicate that a microdose is typically in the range of 5–10% of a full psychedelic dose (Fadiman and Korb, 2019) and that people partake in microdosing for a variety of purposes, including improving cognition and mood, enhancing creativity, or treating health conditions like headaches or neurological problems (Hutten et al., 2019; Lea et al., 2020b; Petranker et al., 2022; Rootman et al., 2021).

Although microdosing is aimed at benefiting health and wellness, there are currently little data to assess the clinical safety profile of chronic microdosing. Most prospective clinical studies of psychedelics have consisted of single doses or multiple doses over a short period of several weeks (Polito and Liknaitzky, 2022). Other studies were observational or retrospective surveys, which have significant limitations for safety assessment (Polito and Liknaitzky, 2022). Furthermore, many different psychedelics, both natural and synthetic, are used for microdosing. Lysergic acid diethylamide (LSD) and psilocybin are by far the most common substances, but surveys show that some respondents use other psychedelics, such as mescaline, N,N-dimethyltryptamine (DMT), and 2C compounds (Cameron et al., 2020; Hutten et al., 2019; Kaertner et al., 2021; Lea et al., 2020a; Petranker et al., 2022; Polito and Stevenson, 2019; Rootman et al., 2021; Rosenbaum et al., 2020). 3,4-methylenedioxymethamphetamine (MDMA) is not a classical psychedelic due to having a distinct mechanism of action and subjective effects (Dunlap et al., 2018), but it has activity at the 5-HT_2 receptors and is also sometimes used in a microdosing fashion (Cameron et al., 2020; Hutten et al., 2019). In addition, there is no set dose or schedule for microdosing. Although one dose every 2 or 3 days is common, reported schedules range from less than once per week to multiple doses per day (Hutten et al.,

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2019; Kaertner et al., 2021; Lea et al., 2020a; Rosenbaum et al., 2020). With this complexity, the safety profiles of all possible microdosing scenarios cannot be easily characterized through clinical studies. Yet, given the number of people who are currently practicing psychedelic microdosing, we must make some safety assessments based on the currently available data to best inform users of possible risks.

One theoretical safety risk of chronic microdosing is the development of valvular heart disease (VHD). VHD can lead to hemodynamic overload on ventricles, with eventual myocardial dysfunction, congestive heart failure, and sudden death (Elangbam, 2010). Drug-induced VHD and primary pulmonary hypertension were first widely recognized following a 1997 report of patients who had been taking the combination of fenfluramine and phentermine (fen-phen) for weight loss (Connolly et al., 1997), although cases had been reported since the 1960s with ergot alkaloids (Elangbam, 2010). Within several years, the mechanism of drug-induced VHD was identified, as all drugs associated with VHD or their metabolites were potent agonists of the serotonin 5-HT_{2B} receptor (Fitzgerald et al., 2000; Rothman et al., 2000). Activation of the 5-HT_{2B} receptor stimulates myofibroblast mitogenesis and extracellular matrix deposition that leads to thickened valve leaflets and the symptoms described above (Elangbam, 2010). The reversibility of these changes remains unclear. Several reports have described specific cardiac changes that persisted for years following the discontinuation of the drug that induced VHD (Dahl et al., 2008; Graham, 1967; Greffe et al., 2007).

This risk of VHD with psychedelic microdosing was first proposed by Kuypers et al. (2019) based purely on receptor interaction profiles (Kuypers et al., 2019). Psychedelics exert their acute subjective effects almost entirely through activation of the serotonin 5-HT_{2A} receptor (Preller et al., 2017; Vollenweider et al., 1998), but many also exhibit high affinity to the structurally similar 5-HT_{2B} receptor. An accompanying commentary by Fadiman and Korb called the worry about VHD "excessive" and predicted that media sites would describe this risk with varying degrees of accuracy (Kuypers et al., 2019). To some extent, this has indeed happened. Various websites have published articles on the cardiac risk of microdosing, including psilocybin, LSD, and MDMA (Smith, 2017; Thomas, 2019, 2022) in addition to mainstream media (Davids Landau, 2022; Eschner, 2022). This risk also continues to be mentioned in academic articles (e.g., Polito and Liknaitzky, 2022).

Despite the potential severity of VHD, the risk of psychedelic and MDMA microdosing has not yet been assessed in a comprehensive manner. Past articles have cited only a limited number of available studies and focused only on LSD and psilocybin. In the current review, we summarize strategies for risk assessment of 5-HT_{2B} agonist-induced VHD at the levels of in vitro, animal, and clinical data. We apply these risk assessments to the most commonly microdosed psychedelics of LSD, psilocybin, mescaline, and DMT, in addition to the non-psychedelic MDMA.

Risk assessment strategies for VHD

Several articles have previously covered safety assessments for 5-HT_{2B} agonists (e.g., Cavero and Guillon, 2014; Papoian et al., 2017). Below, we review how VHD risk can be assessed using in vitro, animal, and clinical studies.

In vitro

Ligand-binding assays are a starting point for assessing the risk of 5-HT_{2B}-mediated VHD. Generally, radioligand-binding assays are utilized, which test the ability of a substance to displace a known radiolabeled ligand from its receptor-binding sites. An inhibition constant (K_i) is calculated from these data which indicates the binding affinity of the ligand for the receptor. If a substance can potently bind to the 5-HT_{2B} receptor, it is necessary to characterize its functional activity using cell-based assays. These experiments will indicate whether the ligand is an agonist and if so, its potency (EC_{50}) and intrinsic activity (E_{max}). In addition to profiling effects at the 5-HT_{2B} receptor, there is an in vitro model of VHD that uses primary cultures of human cardiac valve interstitial cells (hVICs). This model was first used in 2003, where it was demonstrated that known 5-HT_{2B} agonists elicited rapid ERK phosphorylation within 10 min accompanied by a prolonged proliferative response (Setola et al., 2003). Huang et al. also observed a proliferative response to 5-HT_{2B} agonists in 5-HT_{2B}-expressing HEK-293 cells (Huang et al., 2009).

A safety margin can be established by comparing the maximum free plasma level of a drug (C_{max}) to either the K_i or EC_{50} from functional assays (e.g., K_i/C_{max}), with a higher value reflecting a better safety margin. Drug metabolites should be evaluated as well. There are various cases, such as fenfluramine and methysergide, where a metabolite appeared to contribute more to VHD than the parent drug itself (Elangbam, 2010). Although acceptable safety margins have been established for other cardiac targets (i.e., ERG and Nav1.5), there is currently no consensus on what constitutes an appropriate safety margin for 5-HT_{2B} (Papoian et al., 2017). There are various complications to establishing a safety margin for 5-HT_{2B} ligands, as described below.

The 5-HT_{2B} receptor can initiate several intracellular signaling cascades through both G_q proteins and β-arrestin2 (Wang et al., 2021). Activation of G_q stimulates the effector protein phospholipase Cβ (PLCβ). Diacylglycerol (DAG) and inositol triphosphate (IP_3) produced from this increase intracellular calcium ions and activates protein kinase C (PKC) (Wang et al., 2021). Activation of β-arrestin proteins may lead to receptor desensitization, but can also initiate signaling, such as through Akt pathway (Liu et al., 2018).The 5-HT_{2B} receptor may also activate mitogenic pathways through Src kinase and extracellular regulated kinases (ERK) and further enhance the activity of transforming growth factor β (TGF-β) (Wang et al., 2021). Studies of 5-HT_{2B} agonists have used assays for a variety of second messengers, including inositol phosphates, calcium flux, β-arrestin translocation, MAPK2 phosphorylation, and NFAT-bla activity (Cavero and Guillon, 2014; Huang et al., 2009). Agonists of the 5-HT_{2B} receptor exhibit biased signaling, also known as functional selectivity, where certain signaling pathways are activated more strongly than others (Fernandez et al., 2020). For example, LSD can activate the β-arrestin pathway with an EC_{50} approximately 50-fold lower than that for G_q proteins (Wacker et al., 2017).

The functional assay that best predicts VHD risk is not clearly established. One analysis assessed 2200 FDA-approved or investigational medications, including seven known valvulopathogens (Huang et al., 2009). They concluded that calcium flux-based screening was adequate for the initial identification of 5-HT_{2B} receptor agonists, but not discrimination of drugs that have a high

or low risk of VHD. Although the known valvulopathogens all had relatively high potencies across multiple functional assays, no single pattern of functional selectivity appeared to predict VHD risk (Huang et al., 2009). Later analysis showed that the MAPK1/2 and NFAT assays showed greater sensitivity than IP accumulation, calcium flux, and β-arrestin translocation assays (Papoian et al., 2017). However, this same paper concluded that using drug affinity (K_i values) relative to serotonin was a better method of predicting VHD risk than using EC_{50} values from any functional assay (Papoian et al., 2017). One caveat is that the functional assay results came from multiple laboratories with varying experimental conditions that likely resulted in inconsistent EC_{50} values. If functional assays are performed under strict experimental conditions, as described further below, they may have a greater capability of differentiating drugs with risk for VHD.

The results of functional assays can be strongly impacted by both drug-specific factors (Fernandez et al., 2020; Unett et al., 2013) and experimental conditions (Fernandez et al., 2020).The most commonly utilized tissue for both radioligand binding and functional assays is cultured cells transfected with the human version of the 5-HT_{2B} receptor, but transfection can result in receptor expression that is far in excess of physiological levels. Under these conditions, agonists do not need to occupy all receptors to produce a maximum response (Fernandez et al., 2020). The extra unoccupied receptors, referred to as "receptor reserve," can affect EC_{50} values and thus safety margins (Cavero and Guillon, 2014). One example of this was shown with lorcaserin, a drug that was developed for weight loss. Controlling for 5-HT_{2B} receptor expression levels increased the EC_{50} estimates 29-fold (IP accumulation assay) and 3-fold (intracellular calcium assay), without changing the binding affinity (Cavero and Guillon, 2014; U. S. Food and Drug Administration, 2012). Given the lack of standardization of receptor densities, it has also been proposed to use published EC_{50} values relative to those of serotonin to mitigate variability between assay conditions (Papoian et al., 2017). Receptor reserve may cause even greater issues when trying to determine functional selectivity, particularly between pathways that are signal amplifying (e.g., G protein pathways) and non-amplifying (e.g., β-arrestin pathways) (Fernandez et al., 2020).

Animal

Valvular changes are easily missed in the histopathology of chronic toxicology studies in rodents due to anatomy (e.g., thin valve leaflets) and methodological issues (e.g., inconsistent sectioning) (Elangbam, 2010). Thus, standard toxicology studies have missed valvular changes, even when they were present. Histopathologic assessment of heart valves has improved since the risk of 5-HT_{2B} agonists was recognized (e.g., with lorcaserin) (U. S. Food and Drug Administration, 2012).

Attempts have been made to develop a reliable animal model of drug-induced VHD. These include administration of the test drug to adult rodents as well as prenatal administration (Bratter et al., 1999; Donnelly, 2008). Administration of daily subcutaneous serotonin to Sprague Dawley (SD) rats for 3 months resulted in valvular changes that recapitulated those seen in human carcinoid heart disease, both morphologically and on echocardiograms (Gustafsson et al., 2005). Subsequent studies extended this finding to drug-induced VHD. Pergolide administration for 5 months to male Wistar rats induced valvular regurgitation observed on echocardiogram with histopathology that was similar to that of human VHD (Droogmans et al., 2007a, 2009a). Although these results appeared promising, dosing with the known valvulopathogen fenfluramine failed to induce signs of VHD in rats (Therapeutic Goods Administration, 2018). Thus, there is currently no animal model that is considered validated for predicting VHD risk (Elangbam, 2010).

Clinical

The gold standard for assessing drug-induced VHD in human studies is echocardiography. VHD is typically characterized by the thickening of the valvular leaflets, and the thickening and shortening of the subvalvular apparatus of the mitral, aortic, and or less commonly, tricuspid valves, leading to restrictive valve dysfunction (Andrejak and Tribouilloy, 2013; Cosyns et al., 2013; Droogmans et al., 2009b). Coaptation of the damaged valves may eventually lead to regurgitant jets that can be visualized and quantified by color and Doppler echocardiographic assessments.

The most recent example of a comprehensive assessment of clinical VHD risk using echocardiography is lorcaserin. This drug is a selective 5-HT_{2C} agonist which was approved by the FDA in 2012 for weight loss (Miller, 2013), although it was subsequently taken off the market due to the potential for increased cancer risk (de Andrade Mesquita et al., 2021). The FDA had concerns over VHD, despite one animal model showing that a 1400-fold higher dose would be required to stimulate 5-HT_{2B} receptors compared to brain 5-HT_{2C} receptors (Halpern and Halpern, 2015). Thus, echocardiography was performed at multiple time points in all phase 3 studies, including at baseline and weeks 24, 52, 76, and 104 in one study and baseline, 24, and 52 weeks in two others (Halpern and Halpern, 2015). These studies, together totaling 5249 subjects, did not show a significant risk of lorcaserin-induced VHD (Weissman et al., 2013). Even with these results, the FDA label included a warning about the possibility of VHD. In particular, this agency pointed out that safety is unknown in patients with congestive heart failure, where 5-HT_{2B} receptors may be overexpressed, and that lorcaserin should not be used in combination with other 5-HT_{2B} agonists. A post-marketing safety study required by the FDA compared 12,000 patients randomized to lorcaserin or placebo treatment for 1 year and confirmed that there was no greater risk of VHD in those treated with lorcaserin (Bohula et al., 2018).

Clinical studies must have a treatment period long enough to observe VHD. Valvular regurgitation was detected on echocardiography as early as 3 months after fenfluramine use (Jollis et al., 2000). A meta-analysis of fenfluramine studies showed that the risk of valvular regurgitation increased significantly with time up to a period of over 2 years (Hopkins and Polukoff, 2003). Furthermore, any clinical study must be sufficiently powered to detect an increased incidence of VHD or to confirm a lack of increased incidence. There are many drug and study design factors that would affect the ability to detect a signal, particularly the length of the treatment period as mentioned above, the drug pharmacodynamics in terms of the potency, and efficacy for activating the 5-HT_{2B} receptor, drug pharmacokinetics and dosing regimen for determining exposure, and how high plasma levels reach relative to that needed for 5-HT_{2B} activation. In addition,

Table 1. Binding and functional assays with LSD (values in nM).

5-HT_{2A}		5-HT_{2B}		Assay details	Reference
K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)	K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)		
3.5 ([^{125}I]-DOI)	15 [23%] (PI)	30 ([^{125}I]-DOI)		NIH3T3 cells transfected with rat receptors	Nichols et al. (2002)
	21.4 [44%] (Calcium)		8.91 [51%] (Calcium)	CHO-K1 cells transfected with human receptors.	Porter et al. (1999)
0.76 ([^{125}I]-DOI)		0.97 ([^{3}H]-5-HT)		HEK-239 cells transfected with human receptors.	Knight et al., (2004)
11.3 ([^{3}H]-LSD)		30 ([^{3}H]-LSD)		GF62 cells transfected with human receptors.	Ray (2010)
		3.27 ([^{3}H]-LSD)		HEK-293T cells transfected with human receptors.	Wacker et al. (2013)
4.2 ([^{3}H]-ketanserin)	261 [28%] (Calcium)		12,000 [71%] (Calcium)	HEK-293 cells transfected with human receptors.	Rickli et al. (2015b)
3.1 ([^{3}H]-ketanserin)	225 [60%] (Calcium)		207 [13%] (Calcium)	NIH-3 T3 (5-HT_{2A}) or HEK-293 cells (5-HT_{2B}) transfected with human receptors.	Luethi et al. (2019a)
0.33[b] ([^{3}H]-LSD)	3.61 [81%] (Calcium) 0.52 [60%] (β-arrestin2)	0.91[2] ([^{3}H]-LSD)	34 [73%] (Calcium) 0.68 [62%] (β-arrestin2)	HEK-293T cells transfected with human receptors.	Wacker et al. (2017)

Calcium: intracellular calcium assay; PI: phosphoinositol hydrolysis assay.
[a]All E_{max} values are relative to serotonin.
[b]Represents K_d value instead of K_i.

specific populations may be more sensitive to developing drug-induced VHD than others. For example, people with certain cardiac risk factors, conditions that upregulate the 5-HT_{2B} receptor, or who are taking multiple drugs that may interact to increase risk (Droogmans et al., 2009b).

Beyond performing echocardiograms, VHD could potentially be detected through the assessment of adverse events (AEs). Signs and symptoms of VHD include shortness of breath, orthopnea, exercise intolerance, peripheral edema, and palpitations. The main advantage of this approach is that it can be utilized in studies where echocardiography assessments were not performed, including observational and retrospective studies. However, this approach has a number of limitations. Symptomatic VHD is primarily a manifestation of late-stage disease (Iung et al., 2002). Echocardiographic abnormalities were often not accompanied by clinically significant symptoms in drug-induced VHD (Rothman and Baumann, 2009). Additionally, the symptoms of VHD overlap with many other disease processes. There are often differences between studies in how AEs are collected and reported. In many past studies of psychedelics and MDMA, AEs were not systematically assessed (Breeksema et al., 2022). Thus, this approach should not be depended upon by itself for the assessment of VHD risk.

Lysergic acid diethylamide

LSD is a semi-synthetic psychedelic derived from ergot alkaloids that is one of the most common psychedelic substances used for microdosing. In most surveys, about half of respondents who engaged in microdosing reported having used LSD for this practice (Anderson et al., 2019; Hutten et al., 2019; Lea et al., 2020a, 2020b; Petranker et al., 2022; Rosenbaum et al., 2020). A typical LSD microdose is around 10 µg, with a reported range of approximately 5–20 µg (Cameron et al., 2020; Hutten et al., 2019; Kuypers et al., 2019; Lea et al., 2020c; Polito and Stevenson, 2019), which is supported by several clinical studies with dose–response data showing generally subtle effects in this dose range (e.g., Bershad et al., 2019; Greiner, 1958; Holze et al., 2021).

In vitro LSD studies

Studies of the affinity and functional activity of LSD at the 5-HT_{2B} and 5-HT_{2A} receptors are shown in Table 1. LSD affinity for the human 5-HT_{2B} receptor in transfected cell lines was very strong, with K_i or K_d (dissociation constant) values ranging from 0.91 to 30 nM (Knight et al., 2004; Nichols et al., 2002; Wacker et al., 2013, 2017). Binding to the 5-HT_{2A} receptor ranged from being equipotent to about 10-fold stronger than 5-HT_{2B} (Knight et al., 2004; Nichols et al., 2002; Ray, 2010; Wacker et al., 2017).

Several studies have assessed the functional activity of LSD at the 5-HT_{2B} receptor (Table 1). Two groups reported EC_{50} values for the intracellular calcium assay in the low nM range (Porter et al., 1999; Wacker et al., 2017). A third group originally reported an EC_{50} value of 12,000 nM (Rickli, et al., 2015b), but then reported an updated value of 207 nM (Luethi et al., 2019a) after reoptimizing the assay (personal communication with principal investigator Matthias Liechti). The variability in potency estimates between different groups may reflect differences in receptor reserve or other experimental conditions. LSD was a partial agonist with low to moderate intrinsic activity as E_{max} values ranged from 13 to 73%. LSD signaling at the 5-HT_{2B} receptor was strongly biased toward β-arrestin2 over the G_q pathway, as measured by intracellular calcium (Wacker et al., 2013, 2017). Specifically, the EC_{50} value for β-arrestin2 was 0.68 nM, whereas it was 34 nM for calcium influx (Wacker et al., 2017). Signaling

bias in the same direction was seen at the 5-HT_{2A} receptor, where the EC_{50} values were 0.52 and 3.61 nM for the respective signaling pathways.

In vivo LSD studies

Few clinical studies of LSD have been conducted that would be capable of detecting VHD if it were to occur. Most multiple-dose studies of LSD focusing on full psychedelic dosing tested schedules of once every three or four weeks. It is not expected that this infrequent dosing would be capable of inducing VHD. A limited number of studies dosed LSD with more frequent schedules and for periods long enough to potentially observe symptoms of VHD. One study dosed LSD nightly for 36 consecutive nights in 12 subjects (Muzio et al., 1966). In another study, children with schizophrenia ($N=27$) were administered daily LSD (100–150 µg) for periods of between 5.5 and 35 months (Bender, 1966). No cardiac effects were reported in either study, yet no specific safety monitoring was described and the subject numbers were low.

The majority of clinical microdosing studies tested single LSD doses (e.g., Bershad et al., 2019, 2020; Greiner, 1958; Hutten et al., 2020; Murray et al., 2021). Only two multiple-dose studies have been reported, with schedules consisting of a placebo, 5, 10, or 20 µg every 4th day over a 21-day period (Family et al., 2020; Yanakieva et al., 2019) and placebo, 13, or 26 µg LSD tartrate (equivalent to 10 or 20 µg LSD) once every 3 to 4 days for a total of four doses (de Wit et al., 2022). No cardiovascular-related adverse events were reported in either study, and no ECG abnormalities were observed in the study by Family et al. However, several months of chronic dosing is typically needed for VHD symptoms to emerge (Elangbam, 2010), so these dosing periods were too short to assess possible VHD, especially with no echocardiography assessments.

Additional reports of LSD microdosing involved online surveys (Polito and Liknaitzky, 2022), although these have many limitations. Subjects procure their own LSD and so the purity and actual doses taken are typically unknown. Many surveys did not report the length of time that subjects engaged in microdosing. Subjects were mostly current microdosers, thereby missing out on people who stopped microdosing due to side effects. Furthermore, there is no physician monitoring, especially cardiac monitoring, such as an ECG or echocardiogram. Despite these limitations, no symptoms indicative of VHD were reported in these studies.

Animal toxicology data following chronic LSD dosing are limited (Bonson, 2018). One study focused on modeling schizophrenia assessed chronic dosing with a schedule similar to common microdosing schedules (LSD tartrate at 0.08 mg/kg or 0.16 mg/kg i.p. every other day for 3 months, and 0.16 mg/kg every other day for 26 weeks) (Marona-Lewicka et al., 2011). However, a lack of cardiovascular assessments (echocardiogram or histopathology) means that the study did not contribute to knowledge about cardiac safety.

Risk assessment of LSD

There is insufficient in vivo data, either animal or clinical, to assess the risk of VHD with LSD microdosing. Thus, we must rely on calculating safety margins from available in vitro data. The two clinical studies of LSD microdosing that included PK assessments following a 10 µg dose reported a median plasma C_{max} value of 0.323 ng/mL (1.0 nM) (Family et al., 2020) and a geometric mean C_{max} value of 0.279 ng/mL (0.86 nM) (Holze et al., 2021). Considering a free fraction in plasma of 0.1 (Axelrod et al., 1957), the safety margin (K_i/C_{max}) compared to the lowest reported K_i of 0.91 nM is approximately 10. Other compounds known to induce VHD generally had a free C_{max} at least as high as their K_i value for 5-HT_{2B}, if not several-fold higher (Papoian et al., 2017). Thus, LSD microdosing appears to have a relatively low risk.

As demonstrated in the case of fenfluramine, we must also consider the pharmacology of major metabolites. The two main metabolites of LSD are 2-oxo-3-hydroxy-LSD (O-H-LSD) and N-demethyl-LSD (nor-LSD) (Luethi et al., 2019a). The plasma C_{max} of O-H-LSD following a 100 µg LSD dose was 15.5% of its parent (0.11 ng/mL vs. 1.7 ng/mL) (Holze et al., 2019), but it had very limited activity at the 5-HT_{2B} receptor using a calcium assay (Luethi et al., 2019a). Although the in vitro activity of nor-LSD was higher (EC_{50} of 72 nM) (Luethi et al., 2019a), plasma levels of nor-LSD were below the quantifiable limit (<25 pg/mL) following a 100 µg LSD dose (Holze et al., 2019). Thus, neither metabolite is likely to pose much risk considering the extremely low plasma concentrations that would be produced following microdoses.

Psilocybin

Psilocybin is a naturally occurring psychedelic compound found in the fruiting bodies of a variety of mushroom species, mostly of the *Psilocybe* genus; commonly referred to as "magic mushrooms". Psilocybin was the most frequently reported psychedelic substances used for microdosing apart from LSD (Cameron et al., 2020; Hutten et al., 2019; Lea et al., 2020a, 2020b; Petranker et al., 2022; Rootman et al., 2021), with a typical microdose range considered to be approximately 1-3 mg (Hutten et al., 2019; Kuypers et al., 2019; Lea et al., 2020a; Polito and Stevenson, 2019). Psilocybin is a prodrug of psilocin, an active metabolite almost entirely responsible for its psychedelic effects. The parent psilocybin molecule is almost completely metabolized during absorption, and thus is itself not a risk for VHD (Brown et al., 2017; Eivindvik et al., 1989). For this reason, we present in vitro data only for psilocin.

In vitro Psilocin studies

Studies of the affinity and functional activity of psilocin at the 5-HT_{2B} and 5-HT_{2A} receptors are shown in Table 2. Using cell lines with transfected human receptors, psilocin bound to the 5-HT_{2B} receptor with a K_i of 4.6 to 8 nM (Glatfelter et al., 2022; Halberstadt and Geyer, 2011; Ray, 2010). As reported in the same publications, this represented a 23- to 72-fold stronger affinity compared to the 5-HT_{2A} receptor (Glatfelter et al., 2022; Halberstadt and Geyer, 2011; Ray, 2010). Additional studies that assessed binding in the rat and bovine cortex showed the opposite pattern (McKenna and Peroutka, 1989; McKenna et al., 1990), but we do not place much weight on these results considering the different species and potentially non-selective radioligands used.

Table 2. Binding and functional assays with psilocin (values in nM).

5-HT_{2A}		5-HT_{2B}		Details	Reference
Ki (Radioligand)	EC_{50} [E_{max}][a] (Assay)	Ki (Radioligand)	EC_{50} [E_{max}][a] (Assay)		
12 ([^{3}H]-ketanserin)		450 ([^{3}H]-ketanserin)		Rat cortex (2A) and bovine cortex (2B).	McKenna and Peroutka (1989)
6.0 ([^{3}H]-ketanserin)		410 ([^{3}H]-ketanserin)		Rat cortex (2A) and bovine cortex (2B).	McKenna et al. (1990)
	24 [43%] (PI)		58 [45%] (PI)	Cells transfected with human receptors.	Sard et al. (2005)
339.6 ([^{3}H]-LSD)		4.7 ([^{3}H]-LSD)		GF62 cells transfected with human receptors.	Ray (2010)
107.2 ([^{3}H]-ketanserin)		4.6 ([^{3}H]-LSD)		HEK-293 cells transfected with human receptors.	Halberstadt and Geyer (2011)
49 ([^{3}H]-ketanserin)	721 [16%] (Calcium)		>20,000 [ND] (Calcium)	HEK-293 cells transfected with human receptors.	Rickli et al. (2016)
	2.40 [98.4%] (Calcium)		2.37 [39.2%] (Calcium)	Flp-In T-Rex 293 cells transfected with human receptors.	Klein et al. (2021)
	8.34 [82%] (G_q)		1.07 [63%] (G_q)	HEK-293 cells transfected with human receptors	Kargbo (2021); Kozikowski et al. (2020)
180 ([3H]-ketanserin)	13 [67%] (Calcium) 81 [76%] (β-arrestin2)	8 ([3H]-LSD)	8 [38%] (Calcium) 34 [84%] (β-arrestin2)	HEK-293T cells transfected with human receptors	Glatfelter et al. (2022)

Calcium: intracellular calcium assay; G_q: G_q dissociation BRET assay; ND: not determined; PI: phosphoinositol hydrolysis assay.
[a]All E_{max} values are relative to serotonin.

Various functional assays have shown that psilocin is an agonist at the 5-HT_{2B} receptor, with a relatively similar potency to the 5-HT_{2A} receptor. The EC_{50} of psilocin was 58 nM (phosphoinositol hydrolysis assay), 2.37 to 8.0 nM (intracellular calcium assay), and 1.07 nM (G_q dissociation assay), and 34 nM (β-arrestin2 assay) (Glatfelter et al., 2022; Kargbo, 2021; Klein et al., 2021; Kozikowski et al., 2020; Sard et al., 2005). One study reported outlying data showing that psilocin was not an agonist of the 5-HT_{2B} receptor (Rickli et al., 2016). However, as described in the prior section, this group has subsequently optimized their assay, which resulted in significantly stronger potency estimates for LSD. Among the studies showing agonism, psilocin demonstrated moderate intrinsic activity compared to serotonin, with E_{max} values ranging from 38 to 84% (Glatfelter et al., 2022; Kargbo, 2021; Klein et al., 2021; Kozikowski et al., 2020; Sard et al., 2005).

In vivo Psilocybin studies

Clinical studies of low-dose psilocybin consisted of assessing single doses (e.g., Cavanna et al., 2022; Prochazkova et al., 2018) or three weeks of dosing (Marschall et al., 2022; van Elk et al., 2022). One planned study will assess four weeks of dosing 1 mg psilocybin with schedules ranging from twice weekly to three times daily for 5 days per week (NCT04754061, registered at clinicaltrials.gov). Other multiple-dose studies have consisted of medium to high doses administered intermittently every 3 or 4 weeks. None of these studies are sufficient in dosing frequency or duration to adequately assess the risk of VHD.

Similar to the situation with LSD, there have been a number of observational and survey studies (Polito and Liknaitzky, 2022). Although no signals indicating VHD were identified in these studies in terms of subject-reported symptoms, safety monitoring and reporting were most likely inadequate to have captured any signal.

Toxicological studies of psilocybin or psilocybin mushroom extract were generally limited to single-dose studies (e.g., Zhuk et al., 2015). Although studies have been performed with 1 month of dosing (e.g., Huang et al., 2022), cardiovascular endpoints that could demonstrate potential signals of VHD were not performed.

Risk assessment of psilocybin

There are insufficient in vivo data, either animal or clinical, to assess the risk of psilocybin microdosing. Thus, we must rely on the available in vitro data. Psilocybin itself is almost completely metabolized during absorption, and thus is not a risk for VHD by itself (Brown et al., 2017; Eivindvik et al., 1989).

The geometric mean plasma C_{max} value from 15 mg psilocybin was 13 ng/mL of psilocin (63.5 nM) (Holze et al., 2022). Both psilocybin and psilocin have demonstrated dose-proportional exposure, so we may assume that the psilocin C_{max} from the typical microdose of 2 mg is 8.4 nM. The plasma protein binding of psilocin is publicly unknown to the best of our knowledge. The safety margins (K_i/C_{max}) of the total plasma concentrations relative to the lowest reported K_i of 4.6 nM is 0.55. This safety margin by itself is a concern, but even more so considering that the EC_{50} values from some functional assays were actually lower than the reported K_i values.

The main metabolites of psilocin are psilocin glucuronide and 4-hydroxindole-3-acetic acid (4-HIAA). Glucuronide metabolites

Table 3. Binding and functional assays with mescaline (values in nM).

5-HT_{2A}		5-HT_{2B}		Assay Details	Reference
K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)	K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)		
150 ([^{3}H]-ketanserin)		>10,000 ([^{3}H]-ketanserin)		Rat cortex (2A) and bovine cortex (2B).	McKenna and Peroutka (1989)
550 ([^{125}I]-DOI)		795 ([^{3}H]-5-HT)		AV12 cells transfected with human receptors.	Monte et al. (1997)
>10,000 ([^{3}H]-LSD)		793 ([^{3}H]-LSD)		GF62 cells transfected with human receptors.	Ray (2010)
6300 ([^{3}H]-ketanserin)	10,000 [56%] (calcium)		>20,000 [ND] (calcium)	HEK-293 cells transfected with human receptors.	Rickli et al. (2015b)
9400 ([^{3}H]-ketanserin)			>10,000 [ND] (calcium)	HEK-293 cells transfected with human receptors.	Kolaczynska et al. (2022)

Calcium: intracellular calcium assay; ND: not determined.
[a]All E_{max} values are relative to serotonin.

are typically inactive due to steric hindrance of binding to the receptor, although with notable exceptions. The plasma C_{max} of 4-HIAA has been reported as 7 to 18-fold higher than psilocin following an oral dose of psilocybin (Hasler et al., 1997; Kolaczynska et al., 2021). The pharmacology of 4-HIAA at the 5-HT_{2B} receptor remains unknown.

Mescaline

Mescaline (3,4,5-trimethoxyphenethylamine) is a naturally occurring psychedelic molecule that is produced by some members of the *Cactaceae* plant family. Microdosing with mescaline is relatively rare compared to LSD and psilocybin, with only 1.0 to 2.3% of survey respondents reporting that they utilized this substance (Hutten et al., 2019; Kaertner et al., 2021; Lea et al., 2020a; Polito and Stevenson, 2019). An early publication reported the psychedelic dose of mescaline as 180 to 360 mg or higher (Shulgin and Shulgin, 1991) whereas a survey indicated that the typical full psychedelic dose was 400 mg (Hutten et al., 2019). A recent clinical study indicated that the effects of 500 mg of mescaline were comparable to 20 mg psilocybin or 100 µg LSD (Ley et al., 2023). The range of microdoses for mescaline has not been well characterized but is 40 mg if we consider it to be 10% of a standard 400 mg full dose. This is consistent with the microdose reported in one survey of 50 mg (Hutten et al., 2019). Although another reported a lower average microdose of 10 mg, this was based on only two respondents (Polito and Stevenson, 2019). This number is also roughly consistent with a microdosing website that claimed 10 to 40 mg of synthetic mescaline is the typical microdosing range (Third Wave, 2022).

In vitro mescaline studies

There are relatively few experiments that have assessed the affinity and functional effects of mescaline at the 5-HT_{2B} and 5-HT_{2A} receptors (Table 3). The affinity of mescaline was approximately equipotent between the two receptors in one study, but another study showed at least 10-fold more potent binding at the 5-HT_{2B} receptor (Monte et al., 1997; Ray, 2010). A third study showed minimal binding of mescaline to 5-HT_{2B}, but this older study had methodological issues as discussed in prior sections (McKenna and Peroutka, 1989).

Activation of the 5-HT_{2B} receptor using an intracellular calcium assay was not demonstrated with mescaline (Kolaczynska et al., 2022; Rickli et al., 2015b). However, this group has subsequently reoptimized their calcium assay, which resulted in significantly stronger potency estimates of LSD, as described in that section. It is also possible that the potency of mescaline was simply too low to be adequately assessed with the range of concentrations tested. Data by the same laboratory using IP/AA activation assays also indicated that mescaline is equally potent as an activator of the 5-HT_{2A} versus 5-HT_{2B} receptor (personal communication with principal investigator Matthias Liechti). Since psychedelics tend to be biased toward β-arrestin signaling, the potency for activating this pathway could be lower.

In vivo mescaline studies

No clinical studies have been performed with mescaline that would be adequate to assess the risk of VHD. Published (Ley et al., 2023) and ongoing clinical studies (e.g., NCT02033707, NCT04849013 in clinicaltrials.gov) all involve single doses of mescaline. There has also been a study of long-term peyote users (Halpern et al., 2005). However, this study focused on psychological endpoints, and despite the high number of lifetime uses of mescaline by study subjects, the actual dosing frequency was not clear. No animal studies were identified that would allow evaluation of risk for VHD.

Risk assessment of mescaline

There is a lack of clinical and animal studies to assess the VHD risk with mescaline. Recently, the first PK data were published with a validated bioanalytical assay, which quantitated mescaline and its two metabolites 3,4,5-trimethoxyphenylacetic acid (TMPAA) and N-acetyl mescaline (NAM) (Ley et al., 2023; Thomann et al., 2022). The geometric mean plasma C_{max} values for mescaline were 858 ng/mL (3460 nM) and 1217 ng/mL (4900 nM) after doses of 300 and 500 mg

Table 4. Binding and functional assays with DMT (values in nM).

5-HT_{2A}		5-HT_{2B}		Assay details	Reference
K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)	K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)		
42 ([^{3}H]-ketanserin)		550 ([^{3}H]-ketanserin)		Rat cortex (2A) and bovine cortex (2B).	McKenna and Peroutka (1989)
75 ([^{3}H]-ketanserin)		450 ([^{3}H]-ketanserin)		Rat cortex (2A) and bovine cortex (2B).	McKenna et al. (1990)
65 ([^{125}I]-DOI)		101 ([^{3}H]-5-HT)		AV12 cells transfected with human receptors	Blair et al. (1999)
127 ([^{3}H]-LSD)		184 ([^{3}H]-LSD)		HEK-293 cells transfected with human receptors	Keiser et al. (2009)
2323 ([^{3}H]-LSD)		108 ([^{3}H]-LSD)		GF62 cells transfected with human receptors	Ray (2010)
237 ([^{3}H]-ketanserin)	76 [40%] (Calcium)		3400 [19%] (Calcium)	HEK-293 cells transfected with human receptors.	Rickli et al. (2016)

Calcium: intracellular calcium assay
[a]All E_{max} values are relative to serotonin.

mescaline hydrochloride, respectively (Ley et al., 2023). Assuming dose proportionality, a microdose of 40 mg is expected to achieve a plasma C_{max} of approximately 848 nM, which gives a safety margin of 1.07 relative to the lowest reported K_i of 793 nM. This safety margin is concerning, but it is relative to the total plasma concentration instead of the free plasma concentration since plasma protein binding is currently unknown.

The C_{max} values of TMPAA were in a similar range to those of parent mescaline, although C_{max} values for NAM were 20 to 25-fold lower (Ley et al., 2023). The activity of these metabolites at the 5-HT_{2B} receptor has not yet been characterized to the best of our knowledge. TMPAA has been described as non-psychoactive (Dinis-Oliveira et al., 2019) and because potencies at the 5-HT_{2A} and 5-HT_{2B} receptors are highly correlated, it is not likely to have significant potency at the 5-HT_{2B} receptor. Nonetheless, a 40 mg microdose is predicted to achieve a plasma C_{max} of 430 nM for TMPAA, which could result in additional 5-HT_{2B} activation in combination with parent mescaline, even without a very strong potency.

N,N-dimethyltryptamine

DMT is a psychedelic synthesized by a wide variety of plants, but is also found endogenously in humans and other animals (Carbonaro and Gatch, 2016). It has near complete first-pass metabolism by monoamine oxidases (MAOs) and is thus typically inhaled or consumed orally in a brew with other plants containing enzyme inhibitors of MAOs (e.g., ayahuasca) (Barker, 2022). Not every survey reported on the use of DMT, but several have found that it was used by 1.5 to 5.7% percent of people engaging in microdosing, with an additional 0.8 to 1.3% utilizing ayahuasca (Cameron et al., 2020; Hutten et al., 2019; Lea et al., 2020a). Of those who microdosed DMT, it was consumed almost entirely by inhalation (e.g., vaporization or smoking) (Hutten et al., 2020; Lea et al., 2020a). Median DMT doses were in the range of 8 to 10 mg (Hutten et al., 2019; Lea et al., 2020a), compared to a typical full inhaled dose of 40 to 50 mg (Barker, 2022). These reported DMT microdoses are therefore 20% of the full dose, which is notably higher than other commonly microdosed compounds, but may be due to the different route of administration. There has been little experimental study of DMT microdoses, but a dose-ranging study with IV bolus administration showed that 3.75 mg/75 kg DMT fumarate (equivalent of 2.32 mg/75 kg DMT freebase) had mild effects consistent with a typical microdose (Strassman, 1994a). This dose had general relaxing effects without overt changes in perception, and several subjects thought it was a placebo. Given the rapid absorption from the lungs and onset of effects, the PK profile of inhaled DMT is likely to be similar to IV with the exception of incomplete bioavailability. Although one survey reported the typical ayahuasca microdose was 15 mg, two thirds of respondents stated that they did not know the actual dose they were taking (Hutten et al., 2019).

In vitro DMT studies

Studies of the affinity and functional activity of DMT at the 5-HT_{2B} and 5-HT_{2A} receptors are shown in Table 4. DMT exhibited potent binding affinity for the human 5-HT_{2B}, with K_i values ranging from 101 to 184 nM (Blair et al., 1999; Keiser et al., 2009; Ray, 2010). Binding to the human 5-HT_{2A} receptor was about a third more potent compared to 5-HT_{2B} in two publications (Blair et al., 1999; Keiser et al., 2009). A third publication reported that DMT binding to 5-HT_{2B} was about 20-fold more potent than 5-HT_{2A}, but the K_i value for 5-HT_{2A} binding in this study was unusually high compared to others (Ray, 2010).

The functional effects of DMT at the 5-HT_{2B} receptor have not yet been well characterized. A single study reported an EC_{50} value of 3400 nM using the intracellular calcium assay (Rickli et al., 2016), although this assay method has subsequently been optimized, resulting in typically more potent estimates (personal communication with PI Matthias Liechti). The E_{max} value of 19% was notably low compared to the other psychedelics (Rickli et al., 2016), but this needs confirmation using the same assay and further characterization in additional functional assays.

In vivo DMT studies

Several repeat-dose toxicology studies with histological evaluation of the heart have been performed with ayahuasca. A 28-day study of daily oral ayahuasca in male and female rats at doses up to 2-fold the typical full ceremonial dose (0.52 mg/kg DMT) did not result in any histological alterations in the heart (Colaço et al., 2020). An earlier 14-day study of daily oral ayahuasca administered to female rats at up to 50-fold the ceremonial dose (15.1 mg/kg DMT) also did not result in histological alterations (Pic-Taylor et al., 2015). Other repeat-dose toxicology studies have been performed with daily dosing up to 70 days, but did not include histological evaluation (e.g., Santos et al., 2017). Some rodent studies have dosed ayahuasca on intermittent schedules for 2 months and even up to 1 year (e.g., Cameron et al., 2019; Correa-Netto et al., 2017), but did not include cardiac assessments.

Various clinical studies of DMT have been performed, but all were single doses or short series of doses that would not be adequate to assess the risk of VHD (e.g., Good et al., 2023; Riba et al., 2015; Timmermann et al., 2019; Vogt et al., 2023; Strassman, 1994a, 1994b). The health status of long-term ritualistic users of ayahuasca has also been assessed (e.g., Barbosa et al., 2012), but with significant limitations. No studies made specific cardiac assessments relevant to VHD and furthermore, the typical frequency of use was only once every two weeks.

Risk assessment of DMT

There are insufficient in vivo data to fully assess the risk of DMT microdosing. It is promising that 28-day toxicology studies in rats with high DMT doses did not result in histopathological changes to heart valves. However, not all known valvulopathogens induce VHD-like effects in rats (Therapeutic Goods Administration, 2018) and studies that did demonstrate drug-induced VHD-like effects utilized several months of dosing (Droogmans et al., 2007a, 2009a). There are not yet any human studies adequate for assessment of VHD with DMT microdosing.

The PK data following inhaled DMT are very limited (Riba et al., 2015), although the PK of IV administration has been studied fairly extensively in recent years (Good et al., 2023; Timmermann et al., 2019; Vogt et al., 2023). A dose-ranging study showed that a 0.05 mg/kg IV bolus of DMT fumarate resulted in an effect intensity equivalent to a microdose and a plasma C_{max} value of approximately 10 ng/mL (Strassman, 1994a, 1994b). Although DMT microdoses are generally administered in an inhaled fashion, we can assume that users would titrate their dose to a similar C_{max} value. Considering an unbound plasma fraction of 0.677 (Good et al., 2023), the free C_{max} would be 6.8 ng/mL (36 nM). Compared to the lowest reported K_i value of 101 nM (Blair et al., 1999), the safety margin of this unbound C_{max} is 2.8. Although this represents a minimal safety margin, the extremely short half-life of inhaled DMT means that plasma concentrations are only transiently in this range. It is possible that users could compensate for the shorter duration of effect by microdosing DMT more often, but preliminary survey evidence does not indicate that this is the case (Hutten et al., 2020). The mean number of DMT microdoses per week was 2.26, which was not significantly greater than the values for LSD or psilocybin.

Two DMT metabolites have also been assessed in clinical studies. DMT-N-oxide plasma C_{max} values were approximately 60-fold lower than those of parent DMT following an IV bolus dose (Vogt et al., 2023), and so are not likely to represent much risk. Concentrations of indole-3-acetic acid (IAA) can reach much higher levels than DMT (Good et al., 2023; Vogt et al., 2023). However, this molecule is also part of normal tryptophan metabolism and a typical serum level in healthy subjects was reported to be 1.9 μM (Dou et al., 2015).

The PK of full ayahuasca doses has been assessed (dos Santos et al., 2011; Ramaekers et al., 2023), but not that of ayahuasca microdoses. We cannot assume dose-proportionality between full doses and microdoses because not only are DMT doses decreased, but also doses of the enzyme inhibitors of MAO in ayahuasca that permit DMT to escape first-pass metabolism. Although relatively higher doses may be consumed to compensate for this, survey respondents are generally not aware of the DMT doses they are consuming in microdoses of ayahuasca (Hutten et al., 2020). Given these uncertainties, no meaningful analysis of safety margins can be performed at this time with ayahuasca microdoses.

3,4-methylenedioxymethamphetamine

MDMA is not considered a psychedelic but rather falls under a class of drugs called empathogens or entactogens, which are characterized by feelings of euphoria, emotional openness, and connection with others. MDMA produces a distinct pattern from psychedelics on psychometric scales of altered states of consciousness (Studerus et al., 2010). The primary target of MDMA is the serotonin reuptake transporter (SERT), whereby it induces serotonin release from neurons (Dunlap et al., 2018). However, the 5-HT_{2B} receptor may be a secondary target that contributes to serotonin release (Doly et al., 2008). MDMA is worth assessing in this review as 6.5 to 12% of respondents in some online surveys reported microdosing MDMA (Cameron et al., 2020; Hutten et al., 2019), and the potential for the development of VHD following microdosing of MDMA has been mentioned in internet articles (e.g., Smith, 2017). The number of people engaging in microdosing of MDMA compared to psychedelics is not clear since the majority of surveys did not report on MDMA.

A microdose of psychedelics is generally considered to be roughly 10% of the full psychedelic dose. If MDMA were to follow the same pattern, we would expect microdoses of 12.5 to 20 mg based on the full MDMA dose range that is generally between 125 and 200 mg. Popular media articles have claimed that a typical microdose range is 20 to 40 mg (Weiss, 2022) or 5 to 25 mg (Reality Sandwich, 2021). One survey reported the typical MDMA microdose to be 50 mg, although with an extremely broad range of 0.02 to 100 mg (Hutten et al., 2019). It is possible that MDMA microdoses may follow a different pattern compared to psychedelics. Users may not be seeking a true microdose in the sense of being subperceptual, but more of a "mini-dose" (Liechti and Holze, 2022). In addition, MDMA may be unknowingly microdosed as low levels can be added to the mixtures that form various party drugs (Shaw, 2022).

In vitro MDMA studies

Studies of the affinity and functional activity of MDMA and its active N-demethylated metabolite MDA at the 5-HT_{2B} receptor

Table 5. Binding and functional assays with MDMA and MDA (values in nM).

5-HT_{2B}		Assay details	Reference
K_i (Radioligand)	EC_{50} [E_{max}][a] (Assay)		
MDMA = 500 MDA = 100 ([^{3}H]-LSD)	MDMA = 2000 [32%] MDA = 190 [80%] (PI)	HEK-293 cells transfected with human receptors.[b]	Setola et al. (2003)
MDMA = 500 MDA = 91 ([^{3}H]-LSD)		GF62 cells transfected with human receptors.	Ray (2010)
	MDMA > 20,0000[ND] MDA = 850 [52%] (Calcium)	HEK-293 cells transfected with human receptors.	Rickli et al. (2015a)
	MDMA > 10,000 [ND] MDA = 200 [51%] (Calcium)	HEK-293 cells transfected with human receptors.	Luethi et al. (2019b)

Calcium: intracellular calcium assay; P: phosphoinositol hydrolysis assay.
[a]All E_{max} values are relative to serotonin.
[b]Values are from MDMA and MDA as racemic mixtures, although stereoselectivity was demonstrated.

are listed in Table 5. Both bind to the human 5-HT_{2B} receptor, although with a 5-fold lower K_i value for MDA compared to MDMA (Ray, 2010; Setola et al., 2003). Both compounds were agonists in an assay of PI hydrolysis, with MDA (EC_{50} = 190 nM) 10-fold more potent than MDMA (EC_{50} = 2000 nM) in addition to greater intrinsic efficacy (90% vs 32%) (Setola et al., 2003). For comparison, the EC_{50} for the primary mechanism of 5-HT release was 74.3 nM (Baumann and Rothman, 2009). MDA, but not MDMA, also stimulated intracellular calcium release (Rickli et al., 2015a; Luethi et al., 2019b), with the more potent EC_{50} value of the later publication explained by further optimization of this group's assay. Other groups have not reported potency values using the intracellular calcium assay for comparison. Both MDMA and MDA stimulated a mitogenic response at 10 µM in human valvular interstitial cells (Setola et al., 2003).

In vivo MDMA studies

To assess the occurrence of VHD, 29 subjects using or having used MDMA were compared to age and sex-matched controls who did not take MDMA (Droogmans et al., 2007b). The MDMA group had all used MDMA for at least 6 months, with a mean duration of 6.1 years and a mean dose of 3.6 tablets per week. Echocardiography showed that 28% of subjects who took MDMA had signs of VHD compared to no subjects in the control group. An effect of cumulative dose was observed, as subjects with higher grades of regurgitation had taken an average of 943 tablets compared to those with lower grades of regurgitation, who took an average of 242 tablets (Droogmans et al., 2007b). A subsequent case study appeared to confirm that MDMA induced histopathology and echocardiogram findings consistent with drug-induced VHD in a man who self-reportedly took several MDMA pills per week for 16 years (Montastruc et al., 2012). However, there are several limitations to these studies, including that the exact dosing, frequency, and purity of MDMA was unknown.

No animal studies were identified that would be adequate to assess VHD. The closest was a study where the effects of MDMA were tested in rats (dosing 3 consecutive days per week for 6 weeks), but no measures related to VHD, such as echocardiography or heart valve histopathology, were assessed (Jaehne et al., 2008).

Risk assessment of MDMA

Although many unknowns remain, the risk of chronic microdosing with MDMA seems higher than with common psychedelics such as LSD and psilocybin. Retrospective studies appear to show that chronic administration of full MDMA doses resulted in the development of VHD. The risk of regular microdosing is less clear, but may be of clinical relevance given that typical microdoses have been reported to be as high as 50 mg (Hutten et al., 2019).

A 50 mg dose of MDMA resulted in a mean plasma C_{max} 266 nM for MDMA and 28.5 nM for MDA (de la Torre et al., 2000). Free fractions were considered to be 0.57 for MDMA and 0.37 for MDA (Belhadj-Tahar et al., 2010; Wan Aasim et al., 2017). Therefore, the free C_{max} safety margin for MDMA compared to the K_i of 500 nM is 3.29 and the safety margin for MDA compared to the K_i of 91 nM is 8.6.

Other phase I metabolites are formed from MDMA, including 3,4-dihydroxymethamphetamine (HHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA). However, these are rapidly conjugated to sulfates or glucuronides, such that no unconjugated metabolites were detected in human plasma following a 125 mg dose of MDMA (Steuer et al., 2015). Furthermore, the metabolites HHMA, HMMA, HHA, and HMA, did not activate the 5-HT_{2B} receptor at concentrations up to 10 µM (Luethi et al., 2019b). Thus, other metabolites besides MDA can be considered a minimal risk.

Elevated plasma serotonin in combination with 5-HT_{2B} activation may be an additional risk factor for the development of VHD (Rothman and Baumann, 2009). MDMA significantly elevated plasma serotonin levels in rats (Baumann and Rothman, 2009; Yubero-Lahoz et al., 2012; Zolkowska et al., 2006). Although we could not find data on plasma serotonin following MDMA dosing in humans, there are many reported cases of symptoms of serotonin syndrome (Parrott, 2002). Furthermore, if someone does develop VHD, its effects may be compounded by the other cardiac toxicities of MDMA (Bonsignore et al., 2019; Shenouda et al., 2008).

Conclusions

We have summarized the available data for assessing the risk of VHD with four psychedelics and MDMA. All of these

compounds are used for microdosing, where over a long enough period of time, VHD could become a risk if the 5-HT_{2B} receptor is activated. However, this risk assessment should be considered preliminary, as all compounds had at least some missing data that would have significantly improved our analysis.

LSD has a potential risk for VHD considering that the 5-HT_{2B} potency is similar to that of the main target 5-HT_{2A}. Although there is some safety margin, this may not be sufficient considering variability in pharmacokinetics and doses and schedules that are used. Psilocin binds to 5-HT_{2B} much more potently than 5-HT_{2A} and also appears to act as an agonist at 5-HT_{2B}. This indicates it may have a relatively higher risk compared to LSD. Mescaline is not as well characterized because its low potency led to inconclusive results on its actions at the 5-HT_{2B} receptor, but its safety margin based on binding affinity and total plasma concentrations was minimal. DMT also had minimal safety margin, but may be lower risk given its extremely short half-life. MDMA has the highest demonstrated risk of these compounds. Along with its metabolite MDA, it is a potent 5-HT_{2B} agonist with minimal safety margins for reported microdoses and additional factors that increase cardiac risk. Furthermore, VHD appears to have developed in a group of regular long-term users of full MDMA doses, confirming the risk of this compound. Although we have included the most common psychedelics used for microdosing, there are dozens more that are used by a small percentage of people. Each of these compounds has a distinct safety profile, yet little is known about them in order to assess the risk of VHD.

There are a number of caveats and unknowns for calculating the risk of VHD with microdosing. Many microdosing schedules involve every other day or every third day dosing. While this theoretically lowers the VHD risk compared to daily dosing, it is not clear by how much. Almost all known valvulopathogens utilized daily dosing, and thus there is no dataset to evaluate how intermittent dosing affects VHD risk. This question may be addressed using a rat model of VHD.

We are further limited by not knowing which pathway or assay is most related to VHD risk. Psychedelics tend to be strongly biased toward β-arrestin signaling, which may give them a different risk profile compared to other 5-HT_{2B} agonists. The functional assays most strongly associated with VHD were MAPK1/2 phosphorylation and NFAT activation (Papoian et al., 2017), but psychedelics have not yet been assessed using these assays. Furthermore, the functional assays that have been reported were not necessarily performed in systems that mimic physiological receptor expression and could have biased estimates of potency (Cavero and Guillon, 2014). Although we relied heavily on safety margins of binding affinity versus plasma concentrations in our analysis, we must reiterate that there is no established safety margin for VHD. It is impossible at this time to calculate an absolute risk for VHD based on in vitro data alone.

It is notable that out of a large number of people engaging in microdosing, there have not been reports of VHD. However, this is a phenomenon that has only recently gained popularity and it could be that relatively few people have consistently dosed for a long enough period for symptoms of VHD to develop. Although thousands of people have been included in various internet surveys (Polito and Liknaitzky, 2022), these included periods of time that were too short to develop VHD (e.g., Polito and Stevenson, 2019) or in many cases the duration and consistency of microdosing was simply not clear (e.g., Hutten et al., 2019). One survey indicated that 78.5% of respondents had microdosed for up to 6 months, but also did not ask about AEs experienced during this time (Lea et al., 2020b). Furthermore, there are various types of bias inherent to surveys. Thus, although it is encouraging that VHD symptoms have not yet been reported, we do not consider this methodology strong enough to determine that there is no risk.

A number of steps should be taken to better characterize the risk of VHD with psychedelic microdosing. The best way to confirm the safety of microdosing would be a placebo-controlled clinical trial lasting at least 6 months that includes echocardiography assessments. We recognize that the number of different psychedelic substances and microdosing schedules is a complication. Animal studies may be of limited utility for assessing VHD risk considering the questions about their predictive ability. However, replicating and extending in vitro binding and functional assays with 5-HT_{2B} should be a high priority to quickly gather additional information contributing to risk assessment. Cell proliferation assays using hVICs will also be useful considering the uncertainties around which specific assay or pathway is most predictive of VHD.

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References

Anderson T, Petranker R, Rosenbaum D, et al. (2019) Microdosing psychedelics: Personality, mental health, and creativity differences in microdosers. *Psychopharmacology* 236(2): 731–740. DOI: 10.1007/s00213-018-5106-2.

Andrejak M and Tribouilloy C (2013) Drug-induced valvular heart disease: An update. *Arch Cardiovasc Dis* 106(5): 333–339. DOI: 10.1016/j.acvd.2013.02.003.

Axelrod J, Brady RO, Witkop B, et al. (1957) The distribution and metabolism of lysergic acid diethylamide. *Ann NY Acad Sci* 66(3): 435–444. DOI: 10.1111/j.1749-6632.1957.tb40739.x.

Barbosa PCR, Mizumoto S, Bogenschutz MP, et al. (2012) Health status of ayahuasca users. *Drug Test Anal* 4(7–8): 601–609. DOI: 10.1002/dta.1383.

Barker SA (2022) Administration of N,N-dimethyltryptamine (DMT) in psychedelic therapeutics and research and the study of endogenous DMT. *Psychopharmacology* 239(6): 1749–1763. DOI: 10.1007/s00213-022-06065-0.

Baumann MH and Rothman RB (2009) Neural and cardiac toxicities associated with 3,4-methylenedioxymethamphetamine (MDMA). *Int Rev Neurobiol* 88: 257–96. DOI: 10.1016/S0074-7742(09)88010-0.

Belhadj-Tahar H, Payoux P, Tafani M, et al. (2010) Toxicological methods for tracing drug abuse: Chromatographic, spectroscopic and biological characterisation of ecstasy derivatives. *Arh Hig Rada Toksikol* 61(1): 53–59. DOI: 10.2478/10004-1254-61-2010-1937.

Bender L (1966) D-lysergic acid in the treatment of the biological features of childhood schizophrenia. *Dis Nerv Syst* 7: 43–46.

Bershad AK, Preller KH, Lee R, et al. (2020) Preliminary report on the effects of a low dose of LSD on resting-state amygdala functional connectivity. *Biol Psychiatry: Cogn Neurosci Neuroimaging* 5: 461–467. DOI: 10.1016/j.bpsc.2019.12.007.

Bershad AK, Schepers ST, Bremmer MP, et al. (2019) Acute subjective and behavioral effects of microdoses of lysergic acid diethylamide in healthy human volunteers. *Biol Psychiatry* 86: 792–800. DOI: 10.1016/j.biopsych.2019.05.019.

Blair JB, Marona-Lewicka D, Kanthasamy A, et al. (1999) Thieno[3,2-b]- and thieno[2,3-b]pyrrole bioisosteric analogues of the hallucinogen and serotonin agonist N,N-dimethyltryptamine. *J Med Chem* 42: 1106–1111. DOI: 10.1021/jm980692q.

Bohula EA, Wiviott SD, McGuire DK, et al. (2018) Cardiovascular Safety of lorcaserin in overweight or obese patients. *N Engl J Med* 379(12): 1107–1117. DOI: 10.1056/NEJMoa1808721.

Bonsignore A, Barranco R, Morando A, et al. (2019) MDMA induced cardio-toxicity and pathological myocardial effects: A systematic review of experimental data and autopsy findings. *Cardiovasc Toxicol* 19(6): 493–499. DOI: 10.1007/s12012-019-09526-9.

Bonson KR (2018) Regulation of human research with LSD in the United States (1949-1987). *Psychopharmacology* 235(2): 591–604. DOI: 10.1007/s00213-017-4777-4.

Bratter J, Gessner IH and Rowland NE (1999) Effects of prenatal coadministration of phentermine and dexfenfluramine in rats. *Eur J Pharmacol* 369(3): R1–R3. DOI: 10.1016/s0014-2999(99)00100-4.

Breeksema JJ, Kuin BW, Kamphuis J, et al. (2022) Adverse events in clinical treatments with serotonergic psychedelics and MDMA: A mixed-methods systematic review. *J Psychopharmacol (Oxford, England)* 36(10): 1100–1117. DOI: 10.1177/02698811221116926.

Brown RT, Nicholas CR, Cozzi NV, et al. (2017) Pharmacokinetics of escalating doses of oral psilocybin in healthy adults. *Clin Pharmacokinet* 56(12): 1543–1554. DOI: 10.1007/s40262-017-0540-6.

Cameron LP, Benson CJ, DeFelice BC, et al. (2019) Chronic, intermittent microdoses of the psychedelic N,N-dimethyltryptamine (DMT) produce positive effects on mood and anxiety in rodents. *ACS Chem Neurosci* 10(7): 3261–3270. DOI: 10.1021/acschemneuro.8b00692.

Cameron LP, Nazarian A and Olson DE (2020) Psychedelic microdosing: Prevalence and subjective effects. *J Psychoactive Drugs* 52(2): 113–122. DOI: 10.1080/02791072.2020.1718250.

Carbonaro TM and Gatch MB (2016) Neuropharmacology of N,N-dimethyltryptamine. *Brain Res Bull* 126(Pt 1): 74–88. DOI: 10.1016/j.brainresbull.2016.04.016.

Cavanna F, Muller S, de la Fuente LA, et al. (2022) Microdosing with psilocybin mushrooms: A double-blind placebo-controlled study. *Transl Psychiatry* 12(1): 307. DOI: 10.1038/s41398-022-02039-0.

Cavero I and Guillon J-M (2014) Safety pharmacology assessment of drugs with biased 5-HT(2B) receptor agonism mediating cardiac valvulopathy. *J Pharmacol Toxicol Met* 69(2): 150–161. DOI: 10.1016/j.vascn.2013.12.004.

Colaço CS, Alves SS, Nolli LM, et al. (2020) Toxicity of ayahuasca after 28 days daily exposure and effects on monoamines and brain-derived neurotrophic factor (BDNF) in brain of Wistar rats. *Metab Brain Dis* 35(5): 739–751. DOI: 10.1007/s11011-020-00547-w.

Connolly HM, Crary JL, McGoon MD, et al. (1997) Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med* 337(9): 581–588. DOI: 10.1056/NEJM199708283370901.

Correa-Netto NF, Coelho LS, Galfano GS, et al. (2017) Chronic intermittent exposure to ayahuasca during aging does not affect memory in mice. *Braz J Med Biol Res = Revista brasileira de pesquisas medicas e biologicas* 50(7): e6037. DOI: 10.1590/1414-431X20176037.

Cosyns B, Droogmans S, Rosenhek R, et al. (2013) Drug-induced valvular heart disease. *Heart (British Cardiac Society)* 99(1): 7–12. DOI: 10.1136/heartjnl-2012-302239.

Dahl CF, Allen MR, Urie PM, et al. (2008) Valvular regurgitation and surgery associated with fenfluramine use: An analysis of 5743 individuals. *BMC Med* 6: 34. DOI: 10.1186/1741-7015-6-34.

Davids Landau M (2022) Can microdosing psychedelics boost mental health? Here's what the evidence shows. *National Geographic*. Available at: https://www.nationalgeographic.com/science/article/can-microdosing-psychedelics-boost-mental-health-heres-what-the-evidence-shows (accessed 31 January 2023).

de Andrade Mesquita L, Fagundes Piccoli G, Richter da Natividade G, et al. (2021) Is lorcaserin really associated with increased risk of cancer? A systematic review and meta-analysis. *Obes Rev: Off J Int Assoc Stud Obes* 22(3): e13170. DOI: 10.1111/obr.13170.

de la Torre R, Farré M, Ortuño J, et al. (2000) Non-linear pharmacokinetics of MDMA ('ecstasy') in humans. *Brit J Clin Pharmacol* 49(2): 104–109. DOI: 10.1046/j.1365-2125.2000.00121.x.

de Wit H, Molla HM, Bershad A, et al. (2022) Repeated low doses of LSD in healthy adults: A placebo-controlled, dose-response study. *Addict Biol* 27(2): e13143. DOI: 10.1111/adb.13143.

Dinis-Oliveira RJ, Pereira CL and da Silva DD (2019) Pharmacokinetic and pharmacodynamic aspects of peyote and mescaline: Clinical and forensic repercussions. *Curr Mol Pharmacol* 12(3): 184–194. DOI: 10.2174/1874467211666181010154139.

Doly S, Valjent E, Setola V, et al. (2008) Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci: Off J Soc Neurosci* 28(11): 2933–2940. DOI: 10.1523/JNEUROSCI.5723-07.2008.

Donnelly KB (2008) Cardiac valvular pathology: Comparative pathology and animal models of acquired cardiac valvular diseases. *Toxic Pathol* 36(2): 204–217. DOI: 10.1177/0192623307312707.

dos Santos RG, Valle M, Bouso JC, et al. (2011) Autonomic, neuroendocrine, and immunological effects of Ayahuasca. *J Clin Psychopharmacol* 31(6): 717–726. DOI: 10.1097/JCP.0b013e31823607f6.

Dou L, Sallée M, Cerini C, et al. (2015) The cardiovascular effect of the uremic solute indole-3 acetic acid. *J Am Soc Nephrol: JASN* 26(4): 876–87. DOI: 10.1681/ASN.2013121283.

Droogmans S, Cosyns B, D'haenen H, et al. (2007b) Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. *Am J Cardiol* 100(9): 1442–1445. DOI: 10.1016/j.amjcard.2007.06.045.

Droogmans S, Franken PR, Garbar C, et al. (2007a) In vivo model of drug-induced valvular heart disease in rats: Pergolide-induced valvular heart disease demonstrated with echocardiography and correlation with pathology. *Eur Heart J* 28(17): 2156–2162. DOI: 10.1093/eurheartj/ehm263.

Droogmans S, Kerkhove D, Cosyns B, et al. (2009b) Role of echocardiography in toxic heart valvulopathy. *Eur J Echocardiogr: J Work Group Echocardiogr Eur Soc Cardiol* 10(4): 467–476. DOI: 10.1093/ejechocard/jep023.

Droogmans S, Roosens B, Cosyns B, et al. (2009a) Cyproheptadine prevents pergolide-induced valvulopathy in rats: An echocardiographic and histopathological study. *Am J Physiol Heart Circ Physiol* 296(6): H1940–H1948. DOI: 10.1152/ajpheart.01177.2008.

Dunlap LE, Andrews AM and Olson DE (2018) Dark classics in chemical neuroscience: 3,4-methylenedioxymethamphetamine. *ACS Chem Neurosci* 9(10): 2408–2427. DOI: 10.1021/acschemneuro.8b00155.

Eivindvik K, Rasmussen KE and Sund RB (1989) Handling of psilocybin and psilocin by everted sacs of rat jejunum and colon. *Act Pharm Nord* 1(5): 295–302.

Elangbam CS (2010) Drug-induced valvulopathy: An update. *Toxic Pathol* 38(6): 837–848. DOI: 10.1177/0192623310378027.

Eschner K (2022) The promises and perils of psychedelic health care. *The New York Times*, 5 Janauary. Available at: https://www.nytimes.com/2022/01/05/well/psychedelic-drugs-mental-health-therapy.html (accessed 31 January 2023).

Fadiman J and Korb S (2019) Might microdosing psychedelics be safe and beneficial? An initial exploration. *J Psychoact Drugs* 51(2): 118–122. DOI: 10.1080/02791072.2019.1593561.

Family N, Maillet EL, Williams LTJ, et al. (2020) Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers. *Psychopharmacology* 237(3): 841–853. DOI: 10.1007/s00213-019-05417-7.

Fernandez TJ, De Maria M and Lobingier BT (2020) A cellular perspective of bias at G protein-coupled receptors. *Protein Sci: Public Protein Soc* 29(6): 1345–1354. DOI: 10.1002/pro.3872.

Fitzgerald LW, Burn TC, Brown BS, et al. (2000) Possible role of valvular serotonin 5-HT(2B) receptors in the cardiopathy associated with fenfluramine. *Mol Pharmacol* 57(1): 75–81.

Glatfelter GC, Pottie E, Partilla JS, et al. (2022) Structure-activity relationships for psilocybin, baeocystin, aeruginascin, and related analogues to produce pharmacological effects in mice. *ACS Pharmacol Transl Sci* 5(11): 1181–1196. DOI: 10.1021/acsptsci.2c00177.

Good M, Joel Z, Benway T, et al. (2023) Pharmacokinetics of N, N-dimethyltryptamine in humans. *Eur J Drug Metab Pharmacokinet* 48(3): 311–327. DOI: 10.1007/s13318-023-00822-y.

Graham JR (1967) Cardiac and pulmonary fibrosis during methysergide therapy for headache. *Trans Am Clin Climatol Assoc* 78: 79–92.

Greffe G, Chalabreysse L, Mouly-Bertin C, et al. (2007) Valvular heart disease associated with fenfluramine detected 7 years after discontinuation of treatment. *Ann Thorac Surg* 83(4): 1541–1543. DOI: 10.1016/j.athoracsur.2006.11.031.

Greiner T (1958) Psychopathology and psychophysiology of minimal LSD-25 dosage. *A.M.A. Arch Neurol Psychiatry* 79(2): 208. DOI: 10.1001/archneurpsyc.1958.02340020088016.

Gustafsson BI, Tømmerås K, Nordrum I, et al. (2005) Long-term serotonin administration induces heart valve disease in rats. *Circulation* 111(12): 1517–1522. DOI: 10.1161/01.CIR.0000159356.42064.48.

Halberstadt AL and Geyer MA (2011) Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology* 61(3): 364–381. DOI: 10.1016/j.neuropharm.2011.01.017.

Halpern B and Halpern A (2015) Safety assessment of FDA-approved (orlistat and lorcaserin) anti-obesity medications. *Expert Opin Drug Saf* 14(2): 305–315. DOI: 10.1517/14740338.2015.994502.

Halpern JH, Sherwood AR, Hudson JI, et al. (2005) Psychological and Cognitive effects of long-term peyote use among Native Americans. *Biol Psychiatry* 58(8): 624–631. DOI: 10.1016/j.biopsych.2005.06.038.

Hasler F, Bourquin D, Brenneisen R, et al. (1997) Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharm Act Helv* 72(3): 175–184. DOI: 10.1016/S0031-6865(97)00014-9.

Holze F, Duthaler U, Vizeli P, et al. (2019) Pharmacokinetics and subjective effects of a novel oral LSD formulation in healthy subjects. *Br J Clin Pharmacol* 85(7): 1474–1483. DOI: 10.1111/bcp.13918.

Holze F, Ley L, Müller F, et al. (2022) Direct comparison of the acute effects of lysergic acid diethylamide and psilocybin in a double-blind placebo-controlled study in healthy subjects. *Neuropsychopharmacol: Off Public Am Coll Neuropsychopharmacol* 47(6): 1180–1187. DOI: 10.1038/s41386-022-01297-2.

Holze F, Liechti ME, Hutten NRPW, et al. (2021) Pharmacokinetics and pharmacodynamics of lysergic acid diethylamide Microdoses in healthy participants. *Clin Pharmacol Therap* 109(3): 658–666. DOI: 10.1002/cpt.2057.

Hopkins PN and Polukoff GI (2003) Risk of valvular heart disease associated with use of fenfluramine. *BMC Cardiovasc Disord* 3: 5. DOI: 10.1186/1471-2261-3-5.

Huang J, Pham M, Panenka WJ, et al. (2022) Chronic treatment with psilocybin decreases changes in body weight in a rodent model of obesity. *Front Psychiatry* 13: 891512. DOI: 10.3389/fpsyt.2022.891512.

Huang X-P, Setola V, Yadav PN, et al. (2009) Parallel functional activity profiling reveals valvulopathogens are potent 5-hydroxytryptamine(2B) receptor agonists: Implications for drug safety assessment. *Mol Pharmacol* 76(4): 710–22. DOI: 10.1124/mol.109.058057.

Hutten NRPW, Mason NL, Dolder PC, et al. (2019) Motives and side-effects of microdosing with psychedelics among users. *Int J Neuropsychopharmacol* 22(7): 426–434. DOI: 10.1093/ijnp/pyz029.

Hutten NRPW, Mason NL, Dolder PC, et al. (2020) Mood and cognition after administration of low LSD doses in healthy volunteers: A placebo controlled dose-effect finding study. *Eur Neuropsychopharmacol* 41: 81–91. DOI: 10.1016/j.euroneuro.2020.10.002.

Iung B, Gohlke-Bärwolf C, Tornos P, et al. (2002) Recommendations on the management of the asymptomatic patient with valvular heart disease. *Eur Heart J* 23(16): 1253–1266. DOI: 10.1053/euhj.2002.3320.

Jaehne EJ, Salem A and Irvine RJ (2008) The effect of long-term repeated exposure to 3,4-methylenedioxymethamphetamine on cardiovascular and thermoregulatory changes. *Psychopharmacology* 201(2): 161–170. DOI: 10.1007/s00213-008-1258-9.

Jollis JG, Landolfo CK, Kisslo J, et al. (2000) Fenfluramine and phentermine and cardiovascular findings: Effect of treatment duration on prevalence of valve abnormalities. *Circulation* 101(17): 2071–2077. DOI: 10.1161/01.cir.101.17.2071.

Kaertner LS, Steinborn MB, Kettner H, et al. (2021) Positive expectations predict improved mental-health outcomes linked to psychedelic microdosing. *Sci Rep* 11(1): 1941. DOI: 10.1038/s41598-021-81446-7.

Kargbo RB (2021) Improved 5-HT2 selective receptor modulators for the treatment of psychological disorders. *ACS Med Chem Lett* 12(12): 1876–1878. DOI: 10.1021/acsmedchemlett.1c00578.

Keiser MJ, Setola V, Irwin JJ, et al. (2009) Predicting new molecular targets for known drugs. *Nature* 462(7270): 175–181. DOI: 10.1038/nature08506.

Klein AK, Chatha M, Laskowski LJ, et al. (2021) Investigation of the structure-activity relationships of psilocybin analogues. *ACS Pharmacol Trans Sci* 4(2): 533–542. DOI: 10.1021/acsptsci.0c00176.

Knight AR, Misra A, Quirk K, et al. (2004) Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn-Schmiedeb Arch Pharmacol* 370(2): 114–123. DOI: 10.1007/s00210-004-0951-4.

Kolaczynska KE, Liechti ME and Duthaler U (2021) Development and validation of an LC-MS/MS method for the bioanalysis of psilocybin's main metabolites, psilocin and 4-hydroxyindole-3-acetic acid, in human plasma. *J Chromatogr. B, Anal Tech Biomed Life Sci* 1164: 122486. DOI: 10.1016/j.jchromb.2020.122486.

Kolaczynska KE, Luethi D, Trachsel D, et al. (2022) Receptor interaction profiles of 4-Alkoxy-3, 5-dimethoxy-phenethylamines (Mescaline Derivatives) and related amphetamines. *Front Pharmacol* 12: 794254. DOI: 10.3389/fphar.2021.794254.

Kozikowski A, Shapiro G, Tueckmantel W, et al. (2020) 3-(2-(Aminoethyl)-Indol-4-OL derivatives, methods of preparation thereof, and the use as 5-HT2 receptor modulators. WO2021179091. Available at: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2021179091 (accessed 4 April 2023).

Kuypers KPC, Ng L, Erritzoe D, et al. (2019) Microdosing psychedelics: More questions than answers? An overview and suggestions for future research. *J Psychopharmacol* 33(9): 1039–1057. DOI: 10.1177/0269881119857204.

Lea T, Amada N, Jungaberle H, et al. (2020a) Microdosing psychedelics: Motivations, subjective effects and harm reduction. *Int J Drug Policy* 75: 102600. DOI: 10.1016/j.drugpo.2019.11.008.

Lea T, Amada N, Jungaberle H, et al. (2020b) Perceived outcomes of psychedelic microdosing as self-managed therapies for mental and substance use disorders. *Psychopharmacology* 237(5): 1521–1532. DOI: 10.1007/s00213-020-05477-0.

Lea T, Amada N and Jungaberle H (2020c) Psychedelic microdosing: A subreddit analysis. *J Psychoact Drugs* 52(2): 101–112. DOI: 10.1080/02791072.2019.1683260.

Ley L, Holze F, Arikci D, et al. (2023) Comparative acute effects of mescaline, lysergic acid diethylamide, and psilocybin in a randomized, double-blind, placebo-controlled cross-over study in healthy participants. *Neuropsychopharmacology*. Epub ahead of print 25 May 2023. DOI: 10.1038/s41386-023-01607-2.

Liechti ME and Holze F (2022) Dosing psychedelics and MDMA. *Curr Top Behav Neurosci* 56: 3–21. DOI: 10.1007/7854_2021_270.

Liu Y, Wang Z, Li J, et al. (2018) Inhibition of 5-hydroxytryptamine receptor 2B reduced vascular restenosis and mitigated the β-Arrestin2-Mammalian target of rapamycin/p70S6K pathway. *J Am Heart Assoc* 7(3), e006810. DOI: 10.1161/JAHA.117.006810.

Luethi D, Hoener MC, Krähenbühl S, et al. (2019a) Cytochrome P450 enzymes contribute to the metabolism of LSD to nor-LSD and 2-oxo-3-hydroxy-LSD: Implications for clinical LSD use. *Biochem Pharmacol* 164: 129–138. DOI: 10.1016/j.bcp.2019.04.013.

Luethi D, Kolaczynska KE, Walter M, et al. (2019b) Metabolites of the ring-substituted stimulants MDMA, methylone and MDPV differentially affect human monoaminergic systems. *J Psychopharmacol (Oxford, England)* 33(7): 831–841. DOI: 10.1177/0269881119844185.

Marona-Lewicka D, Nichols CD and Nichols DE (2011) An animal model of schizophrenia based on chronic LSD administration: Old idea, new results. *Neuropharmacology* 61(3): 503–512. DOI: 10.1016/j.neuropharm.2011.02.006.

Marschall J, Fejer G, Lempe P, et al. (2022) Psilocybin microdosing does not affect emotion-related symptoms and processing: A preregistered field and lab-based study. *J Psychopharmacol (Oxford, England)* 36(1): 97–113. DOI: 10.1177/02698811211050556.

McKenna DJ and Peroutka SJ (1989) Differentiation of 5-hydroxytryptamine2 receptor subtypes using 125I-R-(-)2,5-dimethoxy-4-iodo-phenylisopropylamine and 3H-ketanserin. *J Neurosci: Off J Soc Neurosci* 9(10): 3482–3490. DOI: 10.1523/JNEUROSCI.09-10-03482.1989.

McKenna DJ, Repke DB, Lo L, et al. (1990) Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology* 29(3): 193–198. DOI: 10.1016/0028-3908(90)90001-8.

Miller LE (2013) Lorcaserin for weight loss: Insights into US food and drug administration approval. *J Acad Nutr Diet* 113(1): 25–30. DOI: 10.1016/j.jand.2012.08.028.

Montastruc F, Montastruc G, Vigreux P, et al. (2012) Valvular heart disease in a patient taking 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). *Br J Clin Pharmacol* 74(3): 547–548. DOI: 10.1111/j.1365-2125.2012.04252.x.

Monte AP, Waldman SR, Marona-Lewicka D, et al. (1997) Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. *J Med Chem* 40(19): 2997–3008. DOI: 10.1021/jm970219x.

Murray CH, Tare I, Perry CM, et al. (2021) Low doses of LSD reduce broadband oscillatory power and modulate event-related potentials in healthy adults. *Psychopharmacology* 239(6): 1735–1747. DOI: 10.1007/s00213-021-05991-9.

Muzio JN, Roffwarg HP and Kaufman E (1966) Alterations in the nocturnal sleep cycle resulting from LSD. *Electroencephalogr Clin Neurophysiol* 21(4): 313–324. DOI: 10.1016/0013-4694(66)90037-x.

Nichols DE, Frescas S, Marona-Lewicka D, et al. (2002) Lysergamides of isomeric 2,4-dimethylazetidines map the binding orientation of the diethylamide moiety in the potent hallucinogenic agent N,N-diethyllysergamide (LSD). *J Med Chem* 45(19): 4344–4349. DOI: 10.1021/jm020153s.

Papoian T, Jagadeesh G, Saulnier M, et al. (2017) Regulatory forum review*: Utility of in vitro secondary pharmacology data to assess risk of drug-induced valvular heart disease in humans: Regulatory considerations. *Toxic Pathol* 45(3): 381–388. DOI: 10.1177/0192623317690609.

Parrott AC (2002) Recreational ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. *Pharmacol Biochem Behav* 71(4): 837–844. DOI: 10.1016/s0091-3057(01)00711-0.

Petranker R, Anderson T, Maier LJ, et al. (2022) Microdosing psychedelics: Subjective benefits and challenges, substance testing behavior, and the relevance of intention. *J Psychopharmacol (Oxford, England)* 36(1): 85–96. DOI: 10.1177/0269881120953994.

Pic-Taylor A, da Motta LG, de Morais JA, et al. (2015) Behavioural and neurotoxic effects of ayahuasca infusion (Banisteriopsis caapi and Psychotria viridis) in female Wistar rat. *Behav Process* 118: 102–110. DOI: 10.1016/j.beproc.2015.05.004.

Polito V and Liknaitzky P (2022) The emerging science of microdosing: A systematic review of research on low dose psychedelics (1955-2021) and recommendations for the field. *Neurosci Biobehav Rev* 139: 104706. DOI: 10.1016/j.neubiorev.2022.104706.

Polito V and Stevenson RJ (2019) A systematic study of microdosing psychedelics. *PLoS One* 14(2): e0211023. DOI: 10.1371/journal.pone.0211023.

Porter RH, Benwell KR, Lamb H, et al. (1999) Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. *Br J Pharmacol* 128(1): 13–20. DOI: 10.1038/sj.bjp.0702751.

Preller KH, Herdener M, Pokorny T, et al. (2017) The fabric of meaning and subjective effects in LSD-induced states depend on serotonin 2A receptor activation. *Curr Biol* 27(3): 451–457. DOI: 10.1016/j.cub.2016.12.030.

Prochazkova L, Lippelt DP, Colzato LS, et al. (2018) Exploring the effect of microdosing psychedelics on creativity in an open-label natural setting. *Psychopharmacol* 235(12): 3401–3413. DOI: 10.1007/s00213-018-5049-7.

Ramaekers J, Mallaroni P, Kloft L, et al. (2023) Altered state of consciousness and mental imagery as a function of N,N-dimethyltryptamine concentration in ritualistic Ayahuasca users. *J Cogn Neurosci* 35(9): 1382–1393. DOI: 10.1162/jocn_a_02003.

Ray TS (2010) Psychedelics and the human receptorome. *PloS One* 5(2): e9019. DOI: 10.1371/journal.pone.0009019.

Reality Sandwich (2021) Common MDMA dosage & microdosing explained. Available at: https://realitysandwich.com/mdma-dosage/#h-what-is-considered-a-microdose-of-mdma (accessed 31 January 2023).

Riba J, McIlhenny EH, Bouso JC, et al. (2015) Metabolism and urinary disposition of N, N -dimethyltryptamine after oral and smoked administration: A comparative study. *Drug Test Anal* 7(5): 401–406. DOI: 10.1002/dta.1685.

Rickli A, Kopf S, Hoener MC, et al. (2015a) Pharmacological profile of novel psychoactive benzofurans. *Br J Pharmacol* 172(13): 3412–3425. DOI: 10.1111/bph.13128.

Rickli A, Luethi D, Reinisch J, et al. (2015b) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). *Neuropharmacology* 99: 546–553. DOI: 10.1016/j.neuropharm.2015.08.034.

Rickli A, Moning OD, Hoener MC, et al. (2016) Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *Eur Neuropsychopharmacol* 26(8): 1327–1337. DOI: 10.1016/j.euroneuro.2016.05.001.

Rootman JM, Kryskow P, Harvey K, et al. (2021) Adults who microdose psychedelics report health related motivations and lower levels of anxiety and depression compared to non-microdosers. *Sci Rep* 11(1): 22479. DOI: 10.1038/s41598-021-01811-4.

Rosenbaum D, Weissman C, Anderson T, et al. (2020) Microdosing psychedelics: Demographics, practices, and psychiatric comorbidities. *J Psychopharmacol* 34(6): 612–622. DOI: 10.1177/0269881120908004.

Rothman RB and Baumann MH (2009) Serotonergic drugs and valvular heart disease. *Exp Opin Drug Saf* 8(3): 317–329. DOI: 10.1517/14740330902931524.

Rothman RB, Baumann MH, Savage JE, et al. (2000) Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* 102(23): 2836–2841. DOI: 10.1161/01.cir.102.23.2836.

Santos A de FA, Vieira ALS, Pic-Taylor A, et al. (2017) Reproductive effects of the psychoactive beverage ayahuasca in male Wistar rats after chronic exposure. *Rev Bras Farmacogn* 27(3): 353–360. DOI: 10.1016/j.bjp.2017.01.006.

Sard H, Kumaran G, Morency C, et al. (2005) SAR of psilocybin analogs: Discovery of a selective 5-HT 2C agonist. *Bioorganic Med Chem Lett* 15(20): 4555–4559. DOI: 10.1016/j.bmcl.2005.06.104.

Setola V, Hufeisen SJ, Grande-Allen KJ, et al. (2003) 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol* 63(6): 1223–1229. DOI: 10.1124/mol.63.6.1223.

Shaw D (2022) Everything you need to know about Tuci- the party drug people are calling 'pink cocaine'. *The Tab*. Available at: https://thetab.com/uk/2022/07/20/everything-you-need-to-know-about-tuci-the-party-drug-people-are-calling-pink-cocaine-263082 (accessed 31 January 2023).

Shenouda SK, Lord KC, McIlwain E, et al. (2008) Ecstasy produces left ventricular dysfunction and oxidative stress in rats. *Cardiovasc Res* 79(4): 662–670. DOI: 10.1093/cvr/cvn129.

Shulgin A and Shulgin AT (1991) *PiHKAL: A Chemical Love Story*, 1st ed. Berkeley: Transform Press.

Smith P (2017) Why microdosing MDMA could be a bad idea. *The Third Wave*. Available at: https://thethirdwave.co/microdosing-mdma/ (accessed 31 January 2023).

Steuer AE, Schmidhauser C, Schmid Y, et al. (2015) Chiral plasma pharmacokinetics of 3,4-methylenedioxymethamphetamine and its phase I and II metabolites following controlled administration to humans. *Drug Metab Dispos: Biol Fate Chem* 43(12): 1864–1871. DOI: 10.1124/dmd.115.066340.

Strassman RJ (1994a) Dose-response study of N,N-Dimethyltryptamine in humans. *Arch Gen Psychiatry* 51(2): 98. DOI: 10.1001/archpsyc.1994.03950020022002.

Strassman RJ (1994b) Dose-response study of N,N-Dimethyltryptamine in humans. *Arch Gen Psychiatry* 51(2): 85. DOI: 10.1001/archpsyc.1994.03950020009001.

Studerus E, Gamma A and Vollenweider FX (2010) Psychometric evaluation of the altered states of consciousness rating scale (OAV). *PloS One* 5(8): e12412. DOI: 10.1371/journal.pone.0012412.

Therapeutic Goods Administration (2018) Australian public assessment report for guanfacine (as hydrochloride). Proprietary Product Name: Intuniv. Sponsor: Shire Australia Pty Ltd. Available at: https://www.tga.gov.au/sites/default/files/auspar-guanfacine-180503.pdf (accessed 4 April 2023).

Third Wave (2022) The ultimate guide to microdosing mescaline. Available at: https://thethirdwave.co/microdosing/mescaline/ (accessed 31 January 2023).

Thomann J, Ley L, Klaiber A, et al. (2022) Development and validation of an LC-MS/MS method for the quantification of mescaline and major metabolites in human plasma. *J Pharm Biomed Anal* 220: 114980. DOI: 10.1016/j.jpba.2022.114980.

Thomas K (2019) Why chronic microdosing might break your heart. *Chacruna*. Available at: https://chacruna.net/why-chronic-microdosing-might-break-your-heart/ (accessed 31 January 2023).

Thomas K (2022) Safety first: Potential heart health risks of microdosing. *Bill of Health*. Available at: https://blog.petrieflom.law.harvard.edu/2022/04/13/safety-first-potential-heart-health-risks-of-microdosing/ (accessed 31 January 2023).

Timmermann C, Roseman L, Schartner M, et al. (2019) Neural correlates of the DMT experience assessed with multivariate EEG. *Sci Rep* 9(1): 16324. DOI: 10.1038/s41598-019-51974-4.

Unett DJ, Gatlin J, Anthony TL, et al. (2013) Kinetics of 5-HT2B receptor signaling: Profound agonist-dependent effects on signaling onset and duration. *J Pharmacol Exp Therap* 347(3): 645–659. DOI: 10.1124/jpet.113.207670.

U. S. Food and Drug Administration (2012) Lorcaserin hydrochloride tablets, 10 mg. Sponsor: Arena pharmaceuticals. *Endocrinologic and Metabolic Drugs Advisory Committee Meeting* – May 10, 2012. Available at: https://www.diabetesincontrol.com/wp-content/uploads/2012/05/www.fda.gov_downloads_AdvisoryCommittees_CommitteesMeetingMaterials_Drugs_EndocrinologicandMetabolicDrugsAdvisoryCommittee_UCM303198.pdf (accessed 4 April 2023).

van Elk M, Fejer G, Lempe P, et al. (2022) Effects of psilocybin microdosing on awe and aesthetic experiences: A preregistered field and lab-based study. *Psychopharmacology* 239(6): 1705–1720. DOI: 10.1007/s00213-021-05857-0.

Vogt SB, Ley L, Erne L, et al. (2023) Acute effects of intravenous DMT in a randomized placebo-controlled study in healthy participants. *Transl Psychiatry* 13(1): 172. DOI: 10.1038/s41398-023-02477-4.

Vollenweider FX, Vollenweider-Scherpenhuyzen MFI, Bäbler A, et al. (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *NeuroReport* 9(17): 3897–3902. DOI: 10.1097/00001756-199812010-00024.

Wacker D, Wang C, Katritch V, et al. (2013) Structural features for functional selectivity at serotonin receptors. *Science (New York, N.Y.)* 340(6132): 615–619. DOI: 10.1126/science.1232808.

Wacker D, Wang S, McCorvy JD, et al. (2017) Crystal structure of an LSD-bound human serotonin receptor. *Cell* 168(3): 377.e12–389.e12. DOI: 10.1016/j.cell.2016.12.033.

Wan Aasim WR, Tan SC and Gan SH (2017) Interspecies in vitro evaluation of stereoselective protein binding for 3,4-methylenedioxymethamphetamine. *J Chem* 2017: 1–7. DOI: 10.1155/2017/8103726.

Wang Q, Zhou Y, Huang J, et al. (2021) Structure, function, and pharmaceutical ligands of 5-hydroxytryptamine 2B receptor. *Pharmaceuticals (Basel, Switzerland)* 14(2): 76. DOI: 10.3390/ph14020076.

Weissman NJ, Sanchez M, Koch GG, et al. (2013) Echocardiographic assessment of cardiac valvular regurgitation with lorcaserin from analysis of 3 phase 3 clinical trials. *Circulation Cardiovasc Imaging* 6(4): 560–567. DOI: 10.1161/CIRCIMAGING.112.000128.

Weiss S (2022) How to microdose drugs based on what you're using. *Vice*. Available at: https://www.vice.com/en/article/xgdpan/what-is-microdosing-psychedelic-mushrooms-lsd-drugs-guide-effects-benefits-risks (accessed 31 January 2023).

Yanakieva S, Polychroni N, Family N, et al. (2019) The effects of microdose LSD on time perception: A randomised, double-blind, placebo-controlled trial. *Psychopharmacology* 236(4): 1159–1170. DOI: 10.1007/s00213-018-5119-x.

Yubero-Lahoz S, Ayestas MA, Blough BE, et al. (2012) Effects of MDMA and related analogs on plasma 5-HT: Relevance to 5-HT transporters in blood and brain. *Eur J pharmacol* 674(2–3): 337–344. DOI: 10.1016/j.ejphar.2011.10.033.

Zhuk O, Jasicka-Misiak I, Poliwoda A, et al. (2015) Research on Acute toxicity and the behavioral effects of methanolic extract from psilocybin mushrooms and psilocin in mice. *Toxins* 7(4): 1018–1029. DOI: 10.3390/toxins7041018.

Zolkowska D, Rothman RB and Baumann MH (2006) Amphetamine analogs increase plasma serotonin: Implications for cardiac and pulmonary disease. *J Pharmacol Exp Ther* 318(2): 604–610. DOI: 10.1124/jpet.106.101618.