Psilocybin Advisory Board Research Subcommittee

**DRAFT OHA Report [Not for Distribution]**

June 22, 2021

1. **Purpose**

The purpose of this rapid review is to provide a focused summary of available evidence and recommendations for development of an Oregon psilocybin services framework to the Oregon Health Authority, as required by Section 11 of Measure 109:

Measure 109, Section 11 (3):

*On or before June 30, 2021, and from time to time after such date, the board shall submit its findings and recommendations to the Oregon Health Authority on available medical, psychological, and scientific studies, research, and other information relating to the safety and efficacy of psilocybin in treating mental health conditions, including but not limited to addiction, depression, anxiety disorders, and end-of-life psychological distress.*

The intent of the rapid review is to highlight particularly pertinent, high quality published works, rather than provide an exhaustive systematic review of the published literature.

1. **Methods**

The Psilocybin Advisory Board Research Subcommittee searched, reviewed, and summarized the available literature on the efficacy and safety of psilocybin to address key questions, which the full board approved on April 28, 2021. The Research Subcommittee conducted the rapid review over eight weeks using the World Health Organization’s rapid review methodology to systematically summarize evidence that informs public policy in a short period of time.1 An experienced research librarian searched Ovid Medline, PsycINFO, and the Cochrane Library, for articles published from inception through May 6, 2021 in Spanish, Russian, German, Danish, English, and Dutch. Specific search terms included psilocybin, psilocin, mushroom, randomized controlled trials, systematic review, meta-analysis, and risk assessment. Full search strategies are available in Appendix X.

The search identified 632 citations. Research subcommittee members reviewed all abstracts and identified relevant articles for full text review (163 articles for Key Questions 1 & 2 and 110 articles for Key Question 4). No relevant publications were identified for Key Question 3. We excluded commentaries and articles that did not involve human subjects, psilocybin, or clinical outcomes.

Published systematic reviews and randomized trials were prioritized for evidence synthesis. Research Subcommittee members supplemented the literature search with additional pertinent peer-reviewed publications when no randomized trials were available and to provide contextual information.

The Oregon Health Authority sought external expert peer review prior to the report’s release on July 30, 2021. A public comment period is scheduled for August 2021.

1. **Key Questions Summary**

The Psilocybin Advisory Board approved the following key questions (KQ) to guide the evidence review:

**KQ1.**  What are the benefits and harms\* of psilocybin in controlled settings in persons seeking treatment for improving condition-specific symptoms and quality of life in the following categories:

a. Depression

b. Anxiety disorders and obsessive-compulsive disorder (OCD)

c. Trauma-related disorders, including racial trauma

d. Substance use disorders

e. Palliative care

f. Spirituality

g. Other conditions

**KQ2.** What are the benefits and harms\* of unsupervised psilocybin use?

*KQ 1 & 2 Sub question:*

Sub question 1: How do the benefits and harms of psilocybin differ by population subgroups, including but not limited to dosage, psilocybin source, age, setting, co-ingestion, or personal characteristics?

**KQ3.** What are provider or patient risk assessment tools that can identify persons likely to benefit or be harmed by psilocybin-assisted therapy?

**KQ4**. What are the relative benefits and harms\* of different sources of psilocybin?

\*includes interpersonal, medical, and psychological harms

1. **Evidence Synthesis**

**KQ1. What are the benefits and harms\* of psilocybin in controlled settings in persons seeking treatment for improving condition-specific symptoms and quality of life in the following categories:**

**a. Depression**

A 2018 systematic review of 10 systematic reviews of trials assessing the effect of psychedelics on mood and anxiety found moderate-to-high quality evidence for the use of psilocybin for treatment of depression and anxiety [Dos Santos Expert Review of Clinical Pharmacology 2018]. Three randomized trials included in these systematic reviews found that psilocybin reduced symptoms of depression in patients with life-threatening diseases, including advanced-stage cancer (92 total participants for at least six months [Grob 2011, Griffiths 2016, Ross 2016 citations].

All trials were conducted in the context of supportive counseling. We identified three additional trials published after this systematic review that confirm and advance these findings. In an open-label, dose-escalating pilot trial of patients with treatment resistant moderate-to-severe major depressive disorder (n=12), depression measured on the Quick Inventory of Depressive Symptoms was reduced at 3 months post-treatment. Sixty-seven percent achieved remission of major depressive disorder at 1 week, and 42% maintained remission at 3 months [Carhart-Harris Lance Psych 2016]. However, this study was considered at high risk of bias due to small sample size, no placebo control/blinding, and no correction for multiple comparisons.

In a randomized crossover trial of two doses of psilocybin (20 mg/70 kg or 30 mg/70 kg) with wait-list controls, participants with moderate-to-severe major depressive disorder (n= 24) experienced reductions in GRID-Hamilton Depression (GRID-HAMD) rating scales that favored the immediate treatment arm with large effect sizes at week 5 (Cohen’s *d*=2.5, p<.001) and week 8 (*d*=2.6, p<.001). Fifty-four percent achieved remission of major depressive disorder at four weeks (moderate risk of bias due to lack of placebo control and blinding [Cite Davis JAMA Psychiatry 2021].

In a phase II, double-blind, randomized trial of psilocybin 25 mg/day versus escitalopram 20 mg/day for 3 weeks for treatment of major depression (n=59), participants in both arms experienced decreases in Quick Inventory of Depressive Symptomatology-Self-Report (QIDS-SR-16) measure of depressive symptoms at 6 weeks with no statistically significant difference between the psilocybin group and the escitalopram group at 6 weeks [Carhart-Harris NEJM 2021].

In a non-blinded, uncontrolled, proof of concept trial (psilocybin 0.3 - 0.36 mg/kg) in men who have sex with men who were long-term AIDS survivors (n=18) with moderate-to-severe “demoralization” (i.e., “poor coping and a sense of helplessness, hopelessness, and a loss of meaning and purpose in life” [citation: Vehling S, Kissane DW, Lo C, Glaesmer H, Hartung TJ, Rodin G, et al. The association of demoralization with mental disorders and suicidal ideation in patients with cancer. Cancer 2017;123(17):3394–401]; stronger association to suicidality than *DSM* Major Depressive Disorder), participants experienced reductions in the self-reported Demoralization Scale-II at the end of treatment and at 3 months (standardized effect size ηp2=.047, 90% CI 0.21-0.60). At end-of-treatment and 3 months, 88.9% and 66.7% of participants, respectively, experienced sustained clinically significant reductions in demoralization [Anderson Eclinical Medicine 2020].

**b. Anxiety Disorders**

A 2018 systematic review of 10 systematic reviews of trials assessing the effect of psychedelics on mood and anxiety found moderate-to-high quality evidence for the use of psilocybin for treatment of depression and anxiety [Dos Santos Expert Review of Clinical Pharmacology 2018]. Three randomized trials included in these systematic reviews found that psilocybin reduced anxiety in patients with life-threatening diseases, including advanced-stage cancer [Grob 2011, Griffiths 2016, Ross 2016 citations].

Grob, et al., conducted a phase I, within-subjects, double-blind, placebo-controlled trial that randomized participants (n=12) with advanced-stage cancer and *DSM-IV* anxiety disorder to receive psilocybin 0.2 mg/kg versus niacin 250 mg as an active control. All participants received both psilocybin and placebo several weeks apart, along with unstructured counseling. Participants experienced no difference in State-Trait Anxiety Inventory score following placebo versus niacin. After dosing with either psilocybin or niacin, State-Trait Anxiety Inventory scores decreased with psilocybin treatment at one and three-month follow-up, but differences were attenuated at six months. Depression, as measured by the Beck Depression Inventory, was improved at six months [Citation: Grob 2011].

A double-blind, placebo-controlled crossover trial tested psilocybin 0.3 mg/kg versus niacin 250 mg (active control) seven weeks apart in participants with life-threatening cancer diagnosis and *DSM-IV* adjustment disorder or generalized anxiety disorder (n=29) [Citation Ross 2016]. All participants received a structured psychotherapy protocol, six hours of preparatory therapy, and 12 hours of integrative therapy, with additional support available from study therapists for 26 weeks after the final study session. Depression scores (Beck Depression Inventory) and anxiety scores (State-Trait Anxiety Inventory) improved following treatment and were sustained at 6.5 months with Cohen’s *d* effect sizes of 0.82 to 1.29. The majority of participants rated the psilocybin experience within the top five most spiritually significant (52%) and personally meaningful (70%) experiences of their lives. The strength of total psilocybin-occasioned mystical-type experience (MEQ30) correlated with greater change in depression and anxiety for most measures [Citation Ross 2016]. Long-term follow-up of, on average, 3.2 years (n=15) and 4.5 years (n=14) indicated statistically significant sustained reductions relative to baseline on all primary measures of anxiety and depression [Agin-Liebes et al., 2020].

Griffiths, et al., conducted a double-blind, placebo-controlled crossover trial of psilocybin 22 or 33 mg/70 kg versus low-dose psilocybin 1 or 3 mg/70 kg (considered a placebo dose) in participants with life-threatening cancer diagnosis and *DSM-IV* anxiety and/or mood disorder (n=51) [Citation: Griffiths 2016]. Each participant received both doses of psilocybin approximately 5 weeks apart, in addition to structured preparatory and integrative psychotherapy. Participants experienced improvements in GRID-Hamilton Depression Rating Scale (GRID-HAM-D-17) and Hamilton Anxiety Rating Scale (HAM-A) after their first psilocybin session with greater effects seen in the high-dose versus low-dose psilocybin groups (Cohen’s *d*=1.30, p<.001 for depression; Cohen’s *d*=1.23, p<.001 for anxiety). Improvements in depression and anxiety remained significant for all participants at 6-month follow-up compared to baseline. At 6-month follow-up, 71% and 63% remained in remission for depression and anxiety, respectively, in the high-dose-first group; while 59% and 50% remained in remission for depression and anxiety, respectively, in the low-dose-first group.

A meta-analysis of the three cancer-related anxiety and depression clinical trials significantly favored psilocybin versus the control group regarding effects on depression (Beck Depression Inventory) and anxiety (State-Trait Anxiety Inventory) [Citation: Vargas et al., 2020].

A small, within-subjects, randomized dose escalation study of psilocybin treatment of people with obsessive compulsive disorder (n=9) documented reductions in the Yale-Brown Obsessive-Compulsive Scale, a measure of OCD symptom severity, 24 hours after psilocybin administration (high risk of bias due to small sample size and short follow-up period). [Cite Moreno 2006]

**c.**  **Trauma-related disorders, including racial trauma**

A high-quality systematic review of psychoactive drugs for the treatment of posttraumatic stress disorder (PTSD) identified no trials of psilocybin for treatment of PTSD [Citation: Varker J Psychoactive Drugs 2021].

There were no trials of use of psilocybin for racial trauma. Published clinical trials of psilocybin included fewer than 10% of participants from under-represented minority groups. A cross-sectional internet-based survey of Black, Indigenous, and people of color in North America who reported a positive experience with psychedelics in the past (n=313), 37% of whom had used psilocybin, asked participants to rate Trauma Symptoms of Discrimination Scale (TSDS) scores before and after their previous psychedelic experiences. Respondents reported reductions in TSDS score following their use of psychedelics. The study has a high risk of bias due to cross-sectional design, potential selection bias, and potential recall bias.

**d. Substance Use Disorders**

Psilocybin has been tested in early phase clinical trials for treatment of tobacco use disorder (one trial) and treatment of alcohol use disorder (one trial).

An early-phase, open-label, uncontrolled, dose-escalation trial of psilocybin (20 mg/70 kg, 30 mg/70 kg, and optional third dose of 20–30 mg/70 kg) combined cognitive behavioral therapy with psilocybin-assisted treatment of tobacco use disorder [Citation: Johnson 2014]. Participants (n=15) smoked an average 19 cigarettes/day (range 15–25) and had an average of 6 unsuccessful previous quit attempts (range 2–12). Eighty percent of participants had confirmed tobacco abstinence at six months. In a follow-up study, 67% were confirmed smoking abstinent at 12 months [Citation: Johnson 2017]. 86.7% rated their psilocybin experiences among the top five most personally meaningful and spiritually significant experiences of their lives, and abstainers scored significantly higher on some measures of the psilocybin-occasioned mystical experience (Garcia-Romeu, 2015). At 16- to 57-month follow-up (n=12), 60% of participants in the original sample were confirmed as smoking abstinent (Johnson, 2016). Participants (n=10) who chose overtone-based music versus Western classical music showed a slight benefit in smoking abstinence (66.7% versus 50%), and psilocybin-occasioned mystical-type experience scores tended to be higher in overtone-based sessions (Strickland, 2020). Analyses of retrospective, semi-structured, follow-up interviews (n=12) identified perceived mechanisms and key themes from these sessions (Noorani, 2018).

An early-phase, open-label, within-subjects, dose-escalation trial of psilocybin (two sessions: 0.3 mg/kg; 0.3–0.4 mg/kg 8 weeks apart) in participants with *DSM-IV* alcohol dependence not currently in treatment (n=10), assessed change in drinking days and heavy-drinking days [Citation: Bogenschutz]. All participants received a structured counseling intervention. Psilocybin treatment was associated with large reductions in the percentage of drinking days (*d*=1.19, p=.007) and percentage of heavy drinking days (*d*=1.38, p=.004) in weeks 25–36 compared with baseline.

**e. Palliative care (pain, end-of-life, etc.)**

Psilocybin has been tested for the relief of depression and anxiety in the context of life-threatening illness. A high-quality systematic review with meta-analysis pooled data from three trials [Citation: Ross 2016, Griffiths 2016, Grob 2011] of psilocybin in people with advance-stage cancer (n=92) [Citation Vargas 2020]. Meta-analysis of these trial data noted large improvements in the Beck Depression Inventory and the State-Trait Anxiety Inventory that favored psilocybin over the control condition. (See Section 4.b. for discussion of individual trials).

Improvements in well-being during life-threatening illness have prompted investigators to call for trials that explicitly test its potential for pain relief [Citation: Whelan and Johnson Pain Management 2018]. We identified one within-subjects pilot randomized trial of psilocybin versus placebo in people with migraine headaches (n=10) [Citation Schindler 2020]. Twenty percent of participants reported at least a 50% reduction following placebo, whereas 50% of participants reported a 50% reduction in weekly migraine days following psilocybin.

Additional clinical trials are currently underway for depression in cancer patients (NCT04593563), and existential distress in palliative care (NCT04754061).

**f. Spirituality**

Hallucinogens have been used for millennia by indigenous cultures for religious ceremonies and mystical rituals (Schultes 1969, 2001, Johnson et al. 2008). Indigenous people with documented usage of hallucinogenic psilocybin species include the Nahuatls, Mayans, Olmec, Mazatecs, Chinantecs, Mixes, Zapotecs, Chatinos, Colima, Purepechas and Totonacs of Mexico [Guzman et al. 2019] and indigenous peoples in parts of Central and South America [Araujo et al. 2015].

One of the proposed mechanisms for observed improvements in depression and anxiety symptoms in clinical trials is a sense of spiritual well-being that many people report during psilocybin treatment. Spiritual phenomenology or mystical experiences in these trials include self-reported experience of meaning beyond oneself and sense of interconnectedness.

A landmark, high quality, double-blinded crossover randomized trial of therapist-facilitated psilocybin (30 mg/70 kg) versus active control (methylphenidate 40mg/70kg) in healthy, hallucinogen-naïve volunteers assessed measures of mystical experience using the Mysticism Scale [Hood J Sci Study Relig 2001] 7 hours and 60 days after ingestion [Griffiths Psychopharmacology 2006]. Volunteers received four preparatory sessions with their therapist before four integration sessions after the day of medication administration. At two months, participants reported experiences of substantial personal meaning and spiritual significance associated with psilocybin exposure. Sixty-seven percent of participants rated their psilocybin experience as the single most or top five most meaningful experience of their lives [Griffiths Psychopharmacology 2006].

A systematic review of psychedelic treatment outcomes identified 10 randomized trials that assessed long-term changes in spirituality after psilocybin use [Aday 2020]. Nine of these 10 trials demonstrated increases in ratings of spiritual well-being from two to 16 months following psilocybin administration. Four of seven trials reporting openness to experiences documented lasting changes in openness [Carhart-Harris et al., 2016b; Erritzoe et al., 2018; Griffiths et al., 2018; Madsen et al., 2020]. One trial reported sustained increases in meditation frequency [Griffiths et al., 2018] and one trial documented increases in mindfulness. [Madsen et al., 2020]

1. **Other Conditions:**

Trials are currently in progress to assess the efficacy of psilocybin for treatment of migraine headache (NCT03341689, NCT04218539), cluster headache (NCT04280055, NCT02981173), post-concussion headache (NCT03806985), short-lasting unilateral neuralgiform headache attacks (NCT04905121), anorexia nervosa (NCT04052568, NCT04505189, NCT04661514), and body dysmorphic disorder (NCT04656301).

**Harms, including interpersonal, medical, and psychological harms:**

Psilocybin is associated with transient negative effects that fall into two main categories: physical and psychological. The best characterization of these transient negative effects is in the clinical trials, many of which are described above, in which they are quantified and frequency and/or severity of the effects is compared to individuals who have received placebo or an active comparator treatment. These effects typically are seen during the administration period. Most effects appear to be dose-dependent—the higher the dose, the more common or intense the negative effect [Dahmane et al, 2021; Carbonaro et al, 2018; Johnson et al, 2012]. Examples of transient negative physical effects include nausea, vomiting, headache, increases in heart rate, increases in blood pressure, and QT interval prolongation (an irregularity in the electrical activity of the heart). [Borowiak et al, 2018; Dahmane et al, 2021; Castro Santos et al, 2021] Psilocybin at a range of doses did not increase body temperature [Hasler et al, 2016]. Examples of transient psychological effects include grief, anxiety or fear, feelings of insanity, feelings of isolation, preoccupation with death, transient thought disorder, and transient paranoia [Carbonaro et al, 2018; Castro Santos et al, 2021; Davis et al, 2021]. Transient negative effects and positive effects (transient and non-transient) can occur during the same administration session [Davis et al, 2020].

Scientific research to date suggests that long-term adverse effects due to psilocybin and other psychedelics are very rare, with the vast majority of clinical trials reporting no long-term adverse effects [Galvao-Coelho et al, 2021; Aday et al 2020; Ross et al, 2016]. Individuals with depression and addiction have specifically noted a subjective lack of long-term adverse effects [ Watts et al, 2017; Noorani et al, 2018]. A small subset of individuals experienced less transient negative effects such as “emotional instability” that resolved within weeks to months [Studerus et al, 2011]. Anxiety and depression that persist well beyond the administration period have been reported in at least two individuals, sometimes ingesting a high dose [Studerus et al, 2011; Benjamin, 1979].

Serotonin syndrome is a toxicity related to consuming one or more drugs that affect serotonin transporters or receptors. Psilocybin acts on serotonin receptors. The risk for this syndrome varies considerably from drug to drug and is highest with combinations of serotonin drugs [Gillman, 2010]. Serotonin syndrome has not been reported in clinical studies with psilocybin and only one article detailing three case reports was found [Suzuki et al, 2016]. Hallucinogen Persisting Perception Disorder (HPPD) has been associated with unsupervised psychedelic use, primarily LSD and cannabis2 [Martinotti et al, 2018]. One case report describes an individual who experienced HPDD after psilocybin and cannabis use [Espiard et al, 2005]. This syndrome has not been reported after supervised clinical use. Psilocybin use in human research settings [Dittrich et al, 1980] and in the community [Nichols, 2016] has not been associated with compulsive, repetitive use.

Many psychedelic experts have emphasized the importance of the context (“set and setting”) of psilocybin administration with respect to some transient negative effects. However, even in tightly controlled research settings, transient psychological manifestations such as anxiety and fear are common [Griffiths et al, 2006]. Pooled data from 23 placebo-controlled studies suggests that psilocybin dose and subject characteristics are the two most critical determinants of the psilocybin experience [Studerus et al, 2012]. Some warn against administering hallucinogens to those having personal or family history of psychotic disorders or other severe psychiatric disorders [Johnson et al., 2008]. In a comparison of the effects of psychedelics (not just psilocybin) and schizophrenia, Leptourgos et al.[2020] found that subjects using hallucinogens can typically recognize distortions in their experience of reality, as opposed to the lack of insight into distortions of reality encountered in schizophrenic psychosis.

Dahmane et al [2021] found in a small group of volunteers that age and body weight had no effect on psilocin area under the curve (AUC)—a measure of total drug exposure, and maximum plasma concentration (Cmax)and suggested that body weight-adjusted dosing is not necessary. The authors suggested 25 mg of psilocybin as a clinical dose at which no clinically significant change in QT interval occurs, while higher doses can result in worsening QT prolongation. Garcia-Romeu et al [2021] reached a similar conclusion on the effect of dose. These dose considerations do not account for repeated microdosing, a practice that might require further study.

It should be noted that much of the research describing transient negative effects is of higher quality, often quantified in the setting of a randomized, controlled clinical trial and in some cases with a placebo or active drug comparator. This strengthens the linkage of these adverse effects to psilocybin and the quantification of their frequency and severity. Much of the research literature regarding more serious adverse events comprises low-quality case reports or descriptions of one or two individuals experiencing these adverse events in the context of a clinical trial. As a result, these events are difficult to definitively link to psilocybin, either because they are rare or because no actual link exists.

Consumption of whole mushrooms may carry additional potential harms. Individuals with fungal allergies are at risk for adverse reactions from whole fungal products. Consuming whole mushroom products poses unique risks, as species of psilocybin-producing fungi vary in the presence and concentration of other bioactive indole alkaloids with structural homology to psilocybin such as baeocystin [Beug et al. 1982, Van Amsterdam et al. 2011, Fricke et al. 2019, Sherwood et al. 2020]. There is variability in presence and abundance of phenylethylalanines in mushrooms which are structural relatives to amphetamines and may induce tachycardia, nausea, and anxiety [Beck et al. 1982]. Other safety considerations during mushroom production include unintentional ingestion due to insufficient personal protective equipment, occupational hazards associated with fungal cultivation and or molecular/biochemical labs. Adverse reactions have also been described by combining psilocybin mushrooms with alcohol, cannabis, cocaine, MDMA, [Skryabin et al. 2011].

**KQ2. What are the benefits and harms of unsupervised psilocybin use?**

No randomized trials assess the benefits and harms of unsupervised psilocybin use. Limited data from observational studies of people regarding unsupervised use suggest that the majority of people using unsupervised psilocybin mushrooms experience subjective benefits and minimal harms. Retrospective studies suggest that the individuals who have consumed psilocybin in the community rarely experience long-term adverse consequences. A review of 6000 psilocybin exposures reported to the National Poison Center between 2000 and 2016 indicated that most calls were from adolescents and young men and were mostly associated with mild and moderate adverse events [Leonard et al, 2018]. A thorough literature review spanning many decades resulted in rare case reports of severe morbidity or mortality associated with unmonitored psilocybin use in the community [Muller et al., 2013; Lim et al., 2012]. Retrospective studies note few fatalities in which psilocybin was believed to be the only drug used, with the few deaths reported usually resulting from events such as drowning or motor vehicle crashes [Leonard et al, 2018]. Circumstances in most cases were poorly characterized.

In a nationally representative U.S. household survey, any lifetime use of psilocybin was associated with decreased adjusted odds of inpatient mental health hospitalization (adjusted odds ratio (aOR)=0.7 [0.5–0.8]), medications for mental health treatment (aOR=0.8 [0.7–0.9]), serious psychological distress (aOR=0.9 [0.8–1.0]), and diagnosis of depression (aOR=0.8 [0.7–1.0]) [citation: Johansen 2015]. In a separate analysis of these data, lifetime psychedelic use, including psilocybin, was associated with reduced odds of past-month psychological distress (weighted odds ratio (OR)=0.81 (0.72–0.91)), past-year suicidal thinking (weighted OR=0.86 (0.78–0.94)), past-year suicidal planning (weighted OR=0.71 (0.54–0.94)), and past-year suicide attempt (weighted OR=0.64 (0.46–0.89)), whereas lifetime illicit use of other drugs was largely associated with an increased likelihood of these outcomes [Citation: Hendricks 2015]. Similarly, any lifetime use of classical psychedelics including psilocybin was associated with a reduced odds of past year larceny/theft (aOR=0.73 (0.65–0.83)), past year assault (aOR = 0.88 (0.80-0.97)), past year arrest for a property crime (aOR=0.78 (0.65–0.95)), and past year arrest for a violent crime (aOR=0.82 (0.70–0.97)), whereas other drug use increased the odds of these outcomes [Citation: Hendricks 2018 ].

Observational studies seeking to assess risks of unsupervised use generally had high risk of bias due to lack of comparison groups or population-based estimates, and cross-sectional study designs. In 346 self-reported psilocybin “bad trips”, females were more represented and the episodes were associated with thinking distortions.[Bienemann 2020] The use of multiple doses of psilocybin in the same session or combining it with other substances was linked to the occurrence of long-term negative outcomes, while the use of mushrooms in single high doses was linked to medical emergencies [Bienemann 2020]. In a web-based survey of people using psilocybin mushrooms (n=1993), participants reporting challenging experiences (i.e. “bad trips”) while taking psilocybin had greater odds of testing positive for neuroticism on the Ten-Item Personality Inventory [Barrett 2017].

***Sub question for KQ 1 & 2:* How do the benefits and harms of psilocybin differ by population subgroups including but not limited to dosage, psilocybin source, age, setting, co-ingestion, or personal characteristics?**

No clinical trials have been conducted specifically to assess the benefits and harms of psilocybin in population subgroups. Reported demographic data about psilocybin indicate that majority of participants are white, college-educated, cis-gender males. Consistent with the early-phase of clinical trials research on psilocybin treatment, most published psilocybin trials exclude patients with comorbid psychiatric and medical conditions. We are consequently unable to comment on differences in psilocybin response by race/ethnicity, gender, or medical subgroups. This limits the generalizability of currently available clinical trials.

Benefits and harms by synthetic versus mushroom-based psilocybin sources are addressed in key question 4. There are no head-to-head clinical trials comparing synthetic to mushroom psilocybin treatment.

**KQ3. What are provider or patient risk assessment tools that can identify persons likely to benefit or be harmed by psilocybin-assisted therapy?**

There are no scientifically validated risk assessment tools for identifying persons with increased likelihood of benefit or harms from psilocybin-assisted therapy. Participants considered at increased risk of mental or physical harm from psilocybin have typically been excluded from clinical trials. Examples include individuals with schizophrenia or heart conditions. Appendix 1 contains example screening considerations along with background information; many of these considerations are directly related to scientifically established harms or potential harms described in KQ1 and KQ2.

**KQ4. What are the relative benefits and harms\* of different sources of psilocybin?**

a **. Fungal physiology, genetics, and identification**

**i. Structure and synthesis of psilocybin**

Psilocybin and the dephosphorylated psychotropic agent psilocin are bioactive indole alkaloids originally derived from fungi. Psilocybin and psilocin and closely related fungal secondary metabolites resulting from coordinated activities of genes which are spatially clustered in fungal genomes [ Reynolds et al. 2018] . The psilocybin production or Psy genes occupy an ~11–22 kilobase genomic region including four genes for synthesis and transport. [ Blei et al. 2018a, 2018b, 2020; Fricke et al. 2018, 2019, 2020, Torrens-Spence et al. 2018, Demmler et al. 2020, Lenz et al. 2021] .

**ii. Identity and species of fungi producing psilocybin**

Psilocybin and psilocin production has been documented in species of the fungal genera *Psilocybe*, *Conocybe*, *Gymnopilus*, *Panaeolus*, *Pluteus*, and *Stropharia* [Reingaridene et al. 2005, Reynolds et al. 2018]. In total, there are over 200 species in over six genera of fungi producing psilocybin and psilocin [Lincoff et al. 1977, Stamets et al. 1996, Guzman et al. 2019]. Some of these species (*P. azurescens, P. stuntzii, P. alennii* and other species that grow on decaying wood) produce chemicals of unknown structure that cause temporary paralysis. While this phenomenon is not yet documented in the primary literature, extreme care should be taken to avoid adverse reactions by consumption of these species.

The wide majority of currently cultivated *Psilocybe* fungi are *P. cubensis.* While species level DNA-based, barcode sequences of the internal transcribed spacer region (ITS) of ribosomal DNA (rDNA) are available in public data repositories such as the National Center for Biotechnology (NCBI) GenBank, many are misidentified and there is a need for the generation of genetic and genomic sequences resources with accurate species level identifications in public databases. Currently there are assembled genomes or raw whole genome and transcriptome data in NCBI databases or *Psilocybe cubensis* (GCA\_017499595.1), *P. cf. subviscida* (GCA\_013368295.1) and *P. cyanescens* (GCA\_002938375.1). The generation of fungal genetic and genomic resources for psilocybin-producing fungi is crucial for their accurate identification.

**iii. Identification of psilocybin producing fungi**

Fungi can be reliably identified to the species level using DNA sequencing by analyzing either genes (short DNA segments) or whole genome sequences (the total DNA in an organism). Further information can be used in concert with molecular DNA data to confidently assign fungal identity including quantifying microscopic morphological observations (Schafer et al. 2000, Uehling et al. 2012), noting species, generic, or familial level characteristics such as spore color in deposit, the presence or absence of tissues such as veils, overall mushroom color, size and stature, morphological patterns of cap or pileal margins (Guzman et al. 2019), and the presence or absence of characteristic blue staining (Reynolds et al. 2018, Lenz et al. 2019). The majority of fungal species that produce psilocybin and psilocin have very visually similar relatives with deadly toxins; misidentification can lead to death (Lincoff et al. 1977). Potential harms of ingesting misidentified fungi include gastrointestinal distress, cellular destruction, liver and kidney damage, autonomous and central nervous system malfunction, and death (Lincoff et al. 1977, Franz et al. 1996). Accurate identification of fungi to species requires molecular DNA sequencing combined with expert evaluation of salient micro- and macromorphological features.

b**. Psilocybin production, extraction, and quantification**

**i. Diversity of psilocybin products** The potential sources for obtaining psilocybin products include (1) *in vivo* cultivation of mushrooms or other naturally occurring fungal tissues such as hyphae or sclerotia; (2) production of psilocybin artificially in cell culture using genetic model organisms [Adams et al. 2019, Milne et al. 2020] or (3) in vitro chemical biosynthesis [Fricke et al. 2019, 2020]. The majority of published or in progress clinical trials utilize synthetic psilocybin [Davis et al. 2021]. Psilocybin products in use differ by region and include a long history of whole mushrooms in Mexico, and Central and South America [Guzman et al. 2008, 2012, 2019], and truffles or sclerotia in Europe [Van Amsterdam et al. 2011]. In addition to these biological products, it has become possible more recently to isolate and purify psilocybin from fungal tissues en masse or from cell cultures [Adams et al. 2019, Milne et al. 2020] and to synthesize psilocybin in vitro [Fricke et al. 2019, 2020]. Accounting for solvents used in extractions and carryover of potentially harmful chemicals or pathogenic microbes (bacteria, viruses, parasites, fungi) from cultivation substrates, especially in compost or dung, will be paramount to ensuring consumer safety. Creating genetically modified microbes that can take residence in the mammalian gut such as *Escherichia coli* or *Saccharomyces cerevisiae* should be considered carefully.

**ii. Psilocybin concentration by product** It has been estimated that fungal tissues differ greatly in psilocybin and psilocin content ranging from ~0.01-2.00% by dry weight [Kamata et al. 2005, Beug et al. 1982].Ingesting 1–4 grams of dried, whole mushrooms, 4–10 mg of pure psilocybin, or 50–300 µg/kg body weight have been considered a dose [Guzman et al. 2014, Beck et al., 1998, Hasler et al., 2004]. The notable variability in psilocybin content from species to species and even between mushrooms in the same fruiting flush [Stribrny et al. 2003, Kamata et al. 2005, Van Amsterdam 2011] coupled with a historical focus on grams of dry weight fungi for ingestion [Guzman et al. 2019] have led to lack of consensus regarding psilocybin dose quantification. Understanding psilocybin concentration as a function of consistent client dosing will be essential to ensuring safe and effective psilocybin treatments.

**iii. Psilocybin extraction and quantification in products** Accurate and reliable quantification of psilocybin and psilocin from fungal tissues or extracts relies on chromatography approaches. Separation and quantification of compounds can be achieved using amino-type polar phase or silica columns combined with reversed-phase liquid chromatography (HPLC) ([Aito et al. 2005, Sottolano et al. 1983, Christiansen et al. 1983, Beug et al. 1981) or with fluorescence (FL) detection (Aito et al. 2005). Products can then be identified via comparison to internal standards, such as 5-methoxytryptamin (Stribrny et al. 2003). Similar chromatographic or mass spec methods can be used to differentiate between fungi that have been counterfeit (impregnated with other hallucinogens such as LSD) with these methods and Thin Layer Chromatography (TLC) (Stahl et al. 1978).

Extraction of psilocybin and psilocin from dried fungal tissues is possible using methanol (Hofmann et al., 1959), and other polar solvents such as water, water-alcohol mixtures, buffer solutions (Van Orden, 2008; Stebelska, 2016). Sonication, maceration, and rotation may affect extraction yields (Van Orden et al. 2008). Qualitative detection of psilocybin and psilocin can be achieved leveraging thin-layer chromatography separations and visualization with Ehrlich’s reagent (Beug and Bigwood et al. 1981). Quantitative psilocybin and psilocin detection methods involve gas chromatography (GC), high-performance liquid chromatography (HPLC), or ultra-high-performance liquid chromatography (UPLC/UHPLC) methods (Stebelska et al. 2016).

Separation of psilocybin and psilocin from fungal tissue homogenate can be carried out using HPLC columns which differentially move cellular contents based on their molecular polarity and result in retention time metrics that are used to identify compounds in complex samples. Normal and reverse phase HPLC differ in the polarity of their stationary and mobile phases and can be adapted to isolate psilocybin and psilocin accordingly (Stebelska et al. 2016). In a related approach called hydrophilic interaction liquid chromatography (HILIC) a hydrophilic stationary phase is combined with reversed mobile phases and results in longer psilocybin and psilocin retention times. Utilizing larger or longer columns and combining columns can differentiate between psilocybin, psilocin, and other related highly polar compounds based on their retention times (Nagy and Veress, 2016; Rácz et al., 2018; Veress et al., 2019).

Detection of psilocybin and psilocin can be achieved by combining HPLC systems with either an ultra-violet/visible light spectroscopy detector (HPLC-UV/Vis) or diode array detectors (DAD). Psilocybin concentration data can be derived from these analyses by quantifying the amount of specific wavelength UV or visible light absorbed by a molecule. A second approach for detection of psilocybin, psilocin, and related compounds is by coupling HPLC systems with mass spectrometers (HPLC-MS). Molecules of interest are first filtered and then collided with an inert gas in a collision cell to yield daughter ions as fragments of the initial mass. The resulting molecular fingerprints can be used to precisely identify psilocybin, psilocin, and potential contaminants in samples (Van Orden et al. 2008, Stebelska et al. 2016, Nagy et al. 2016, Veress et al. 2019).

Quantification of psilocybin, psilocin, and other compounds is achieved by comparing experimental samples to a calibration curve of data from pure analytes (psilocybin or psilocin) prepared at a range of known concentrations. To avoid quantification artifacts related to chemical interactions, an internal standard such as deuterated psilocybin, psilocybin-d4 (Zhou et al. 2021) or synthetic indolealkylamine derivatives and structural isomers (e.g. Anastos et al., 2006b; Gambaro et al. 2015) can be included in experimental samples.

To ensure product safety, it will be critical to assess contaminants that could arise during product manufacture or cultivation. Concerning contaminants include (1) residual solvents and/or disinfectants used for sterilization or involved in the extraction process, (2) toxic metals, pesticides, antibiotics, herbicides, animal husbandry medications, and bioaccumulated from contaminated growth substrates, (3) microbiological concerns in the form of both other fungi and bacteria, and (4) insecticides, antibiotics, or other pesticides which may be applied directly to fungi in an attempt to limit the presence of flies and mites

Mushrooms and fungal tissues are ephemeral structures prone to microbial, insect-related and arachnid-related decay. As such, screening for these pathogens and chemicals used to treat infestations of manufacture operations must be considered. Common mushroom contaminants that can be screened for include species of the fungal genera *Trichoderma* (green mold), *Aspergillus*, *Dactylium*, *Lecanicillium*, *Mucor, Rhizopus, Mycogone*, *Neurospora,* and *Penicillium* (Stamets and Chilton, 1983). Bacteria that affect mushrooms and fungal cultures include species of the genera *Pseudomonas* and *Ewingella* and others (Stamets and Chilton, 1983). Insect and arachnid pathogens of mushrooms include species of the genera *Lycoriella*, *Megaselia*, *Heteropeza, Mycophila, Leptocera, Tyrophagus, Caloglyphus, Linopodes, Tarsonemus, and Pygmephorus* (Stamets et al. 1983).

The presence and quantity of contaminants including residual solvents from extractions and disinfectants from cultivation (Lambert, 1938; Thomas et al., 1956; Stamets and Chilton, 1983; Moore, 2005) can be evaluated using GC-MS, HPLC-MS, or HPLC-UV/Vis. Bioaccumulated heavy metals can be detected and quantified using atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), x-ray fluorescence (XRF), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) or quadrupole ICP-MS (Bressa et al., 1988; Gabriel et al., 1996; Tüzen et al., 1998; Michelot et al., 1998; Falandysz et al., 2001; Demirbaş, 2001a, 2001b; Demirbaş, 2002; Ángeles García et al., 2009; Gabriel et al., 2016; Širić et al., 2016; Tel-Çayan et al., 2018; Sener, 2019). Residual pesticides in mushrooms or hyphae can be detected using GC-MS, GC-MS/MS, HPLC-MS, HPLC-MS/MS, UPLC-MS, UPLC-MS/MS, and hybrid quadrupole-Orbitrap HPLC systems (Chang et al. 2014; Gałgowska et al. 2017; Du et al. 2018; Tian et al. 2020).

1. Tricco AC, Langlois EV, Straus SE, editors. Rapid reviews to strengthen health policy and systems: a practical guide. In. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO; 2017.

2. Martinotti G, Santacroce R, Pettorruso M, et al. Hallucinogen Persisting Perception Disorder: Etiology, Clinical Features, and Therapeutic Perspectives. *Brain Sci.* 2018;8(3).