JHM IRB - eForm A – Protocol

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

1) Tetrahydrocannabinol (THC) has been the primary focus of cannabis research since its isolation and synthesis in 1964 (Gaoni and Mechoulam 1964). Currently there is a lot of debate regarding whether, and which, non-THC constituents of the cannabis plant contribute to the overall pharmacodynamic effects of cannabis. In recent publications, Ethan Russo has hypothesized that specific cannabis terpenoids, aromatic essential oil (EO) components, may selectively mitigate or exacerbate some acute effects of THC (McPartland and Russo 2001, Russo 2011, McPartland and Russo 2014). This purported "entourage effect" has been the driving force behind state legalization of cannabis for medicinal purposes despite THC (as dronabinol) being available as an FDA approved medication. However, there have been no empirical research studies conducted to evaluate the interactions of THC and terpenoids found in the cannabis plant. The proposed study will be a controlled human laboratory evaluation of the acute dose effects of THC and d-limonene (limonene) alone and in combination. Limonene is a flavor and fragrance component common to many plants, is one of the most abundant terpenoids in cannabis, is a fragrance ingredient in many household products, and is part of the typical human diet as it is present in most citrus fruits. Oral ingestion of d-limonene is Generally Recognized as Safe (GRAS) by the US Food and Drug Administration and other regulatory agencies, and studies have been conducted evaluating its effects when inhaled in ambient air. Prior research has demonstrated that limonene inhaled from ambient air can produce anxiolytic effects and reduce depression (Buchbauer et al. 1993, Carvalho-Freitas and Costa 2002a, Pultrini Ade, Galindo, and Costa 2006, Falk-Filipsson et al. 1993). This study will evaluate whether limonene, compared with placebo, attenuates increases in anxiety that often occur following acute THC administration. A controlled laboratory study will be conducted with healthy adults who have experience inhaling cannabis. Participants will complete 9 acute drug administration sessions at the Behavioral Pharmacology Research Unit (BPRU). Sessions will involve double blind administration of: 1) Placebo, 2) 15mg THC, 3) 30mg THC, 4), 1mg d-limonene, 5) 5mg d-limonene, 6) 15mg THC + 1mg d-limonene, 7) 30mg THC + 1mg d-limonene, 8) 15mg THC + 5mg d-limonene, and 9) 30mg THC + 5mg d-limonene via vaporization. Participants who complete these 9 primary conditions, and who exhibit increased anxiety after THC exposure, will be invited to complete an optional 10th study condition in which they administer 30mg THC + 15mg d-limonene to determine whether a higher dose of d-limonene can further attenuate the anxiogenic effects of THC than what is observed at the 1mg and 5mg doses. At baseline and following drug administration, a battery of subjective, physiological, and cognitive performance assessments will be completed and biological specimens obtained. The study will conclude when 20 participants complete all 9 primary experimental sessions. The outcomes of this study will be

useful to inform clinical decision making and policy regarding the use of cannabis versus dronabinol, and will provide needed empirical data to either support or refute claims made in the cannabis industry that limonene mitigates the anxiogenic effects of high doses of THC.

2. Objectives (include all primary and secondary objectives)

Objective 1: Examine the pharmacodynamics and pharmacokinetics of vaporized THC and d-limonene alone and in combination.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Delta-9-tetrahydrocannabinol (THC) is the primary psychoactive chemical constituent of the cannabis plant. The effects of THC have been well characterized in controlled research. Positive and/or therapeutic effects include feelings of euphoria, relaxed mood, enhanced enjoyment of music/art, as well as analgesic, anti-inflammatory, hypnotic, muscle relaxant, bronchodilatory, antiemetic, and appetite stimulant effects. Negative or unwanted side effects include dysphoria (panic, paranoia, acute psychosis), nausea/emesis dry mouth, irritated eyes, hallucinations, and cognitive impairment (working memory, divided attention, time estimation, complex cognition). These effects are produced through a combination of partial agonism at the CB_1 and CB_2 receptors, as well as non-receptor mechanisms (Russo 2011). For many, THC is synonymous with cannabis, and, over the past 30 years, illicit drug producers have selectively bred cannabis plants to contain ever-greater concentrations of THC, which now accounts for 15-25% of the dried flowers of the plant sold to consumers in the U.S. Also, with the advent of a legal medicinal and non-medicinal cannabis market in over half the U.S. states, a larger emerging product market of “concentrates” has developed. In production of these products, THC is extracted from the plant material resulting in a resin that contains 75-90% THC. Recently, a trend has emerged among manufacturers to “spike” these cannabis resins with select terpenoids or terpenoind combinations as a means of producing tailored pharmacodynamic effects.

A key controversy in understanding the pharmacology of cannabis is whether its behavioral and psychoactive effects are wholly accounted for by THC, or whether, in contrast, other cannabis components including “minor” cannabinoids (e.g. cannabidiol, cannabinol, cannabigerol) and terpenoids substantively influence its effects. Part of the problem has been compounded by the fact that cannabis supplied for experimentation in the USA is notably deficient in minor cannabinoid and terpenoid content as compared to that available via the black market where plants are selectively bred to have specific cannabinoid and terpenoid profiles based on the belief that certain ratios confer different effects on the user (Bloor et al. 2008). These beliefs, however, are based largely on anecdote, with little controlled research to inform the interaction of THC and minor cannabinoids and no published human research on the interaction between THC and terpenoids.

Terpenoids are produced in glandular trichomes of cannabis along with phytocannabinoids, and are pharmacologically versatile: interacting with cell membranes, neuronal and muscle ion channels, neurotransmitter receptors, G-protein coupled (odorant) receptors, second messenger systems and enzymes (Bowles 2003, Buchbauer 2010). D-Limonene is common to the lemon and other citrus essential oils (EOs) and is the second most widely distributed terpenoid in nature (Noma and Asakawa 2010). D-Limonene is also one of the most abundant terpenoids found in the cannabis plant. It is a fragrance ingredient in many household products, and is part of the typical human diet as it is present in most citrus fruits and oral ingestion of d-limonene is Generally Recognized as Safe (GRAS) by the US Food and Drug Administration and other regulatory agencies. Studies of citrus oils in mice suggest d-limonene produces anxiolytic effects (Carvalho-Freitas and Costa 2002b). The mechanism of these effects is hypothesized to be increased serotonin in prefrontal cortex (PFC), and dopamine (DA) in hippocampus mediated via $5\text{-}HT_{1A}$ receptors (Komiya, Takeuchi, and Harada 2006). Human research on the anxiolytic effects of inhaled d-limonene has been limited to a single study (Komori et al. 1995), in which hospitalized depressed patients were exposed to citrus fragrance in ambient air. In that study, Hamilton Depression Scores (HADS) were reduced by the citrus fragrance, 9/12 patients discontinued

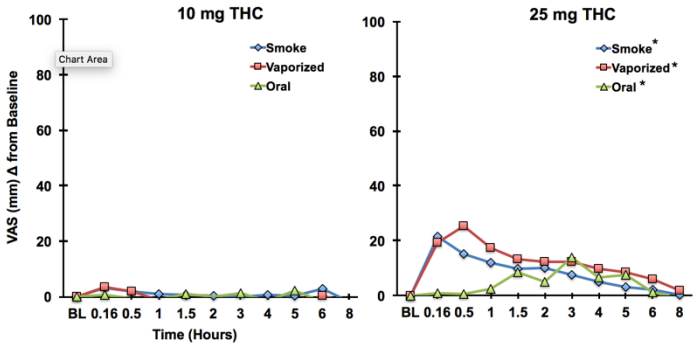
antidepressant medication, and serum evidence of immune stimulation (CD4/8 ratio normalization) was observed. Inhalation of d-limonene has high bioavailability, with an estimated 70% human pulmonary uptake, and was well tolerated in a controlled exposure study (Falk-Filipsson et al. 1993). D-limonene produces a hepatic metabolite, perillic acid, which demonstrates stress reduction effects in the rat brain (Fukumoto et al. 2008). D-Limonene is non-toxic at low doses (estimated human lethal dose 0.5-5 g/kg^{-1}) and non-sensitizing (Von Burg 1995), and an estimate of exposure to limonene for the general population via indoor air is 10g/kg/day (Kim et al. 2013).

Currently, there is rapidly growing interest in the development of cannabis and cannabinoid based medicines for the treatment of myriad health conditions. THC (dronabinol) has been approved by the FDA for 30 years, yet is rarely used in medicine. Part of the reason for that is because the therapeutic index of pure THC, when given intravenously (D'Souza et al. 2004) or orally (Favrat et al. 2005) is narrow, especially among individuals previously naïve to its effects. Acute overdose incidents involving THC or THC-predominant cannabis commonly include anxiety, panic reactions or toxic psychoses, for which no pharmacological intervention is generally necessary, but which can result in significant and sustained discomfort to the individual. In recent years, advocates for cannabis legalization have argued that the use of cannabis (versus pure THC) reduces the rate and severity of adverse effects in the treatment of medical conditions, and this argument has been the basis for establishing medicinal cannabis laws in 29 of the 50 U.S. states and the District of Columbia.

To date, scientific evaluation of the “entourage” effects of cannabis versus THC alone have been largely limited to the study of cannabidiol (CBD) as a compound purported to attenuate some of the untoward psychoactive effects of THC and generally reduce its adverse event profile. Clinical research by a UK pharmaceutical company suggests that producing cannabinoid medicine with a 1:1 ratio of THC:CBD produces better clinical results with fewer side effects relative to higher THC:CBD ratios (Russo and Guy 2006). However, laboratory studies have failed to detect a modulatory effect of CBD on the subjective effects of THC (Ilan et al. 2005, Haney et al. 2016). Though there is a lack of controlled research, numerous reports in published literature dating back to the 10th century indicate that the consumption of lemons, lemonade, citrus fruits, pine nuts, calamus root, or other natural sources of d-limonene or related substances can have the effect of an “antidote” to harms or adverse effects associated with the consumption of cannabis or hashish.

The aim of the proposed study is to evaluate, in a controlled laboratory experiment, whether and to what degree, d-limonene modulates the acute effects of inhaled THC. The procedures being used have been established in our lab during recent studies evaluating the dose effects of cannabis administered via oral ingestion, smoke, or vaporization. Results of those studies demonstrate that we can reliably produce and validly measure acute cannabis effects and were used to inform the THC doses selected for this study. We safely administered vaporized cannabis containing THC doses of 10mg and 25mg to healthy adult volunteers in a recent study (IRB00035394). These doses produced dose-orderly drug effects. The 10mg THC dose was not associated with an increase in anxiety and produced mild to moderate drug effects and the 25mg THC produced mild to moderate anxiety in a subset of participants (see Figure below). We believe that increasing the doses to 15mg and 30mg in this study will increase the likelihood of anxiety following dosing as well as the severity of anxiety after dosing in study participants, but will remain tolerable.

Anxious/Nervous



The primary doses of d-limonene to be used in this study (1mg and 5mg) are within the range of what would be inhaled by an individual who smokes 1 gram of cannabis. In recent research studies conducted in our laboratory, 1 gram is the amount of plant material commonly used to make a single cannabis cigarette ("joint" or "blunt"). In addition, chemical analysis of 107 samples of cannabis consisting of 29 different "strains" of cannabis produced by a Canadian medicinal cannabis manufacturer indicate that 1 mg would be the median and 5mg would be the maximum d-limonene dose in a 1 gram cannabis cigarette produced with their products. Thus, these doses are ecologically relevant and have demonstrated safety with respect to acute dosing via direct inhalation. The optional 15mg dose of d-limonene is higher than what would typically be consumed in natural botanical cannabis, but should not present any risk of harm. A current practice in the cannabis industry is to supplement formulated cannabis products with extracted terpenes. Further evidence for the safety of d-limonene comes from a prior study (Falk-Filipsson et al., 1993) in which healthy adults were continuously exposed to high levels of d-limonene in an enclosed chamber for 2 hours; in this study, participants absorbed up to about ~26 mg of d-limonene without experiencing adverse events or irritative symptoms. At the time that the amendment to this protocol adding the 15mg d-limonene dose was submitted, no adverse effects had been reported following either 1mg or 5mg d-limonene administration for the first 16 randomized participants. The results of this study will represent a significant initial advancement in understanding the behavioral pharmacology of cannabis. This is the initial study in a series of similar experiments funded by NIH in which we will begin to systematically evaluate the interactions of multiple components of the cannabis plant. Characterization of the interaction between THC and d-limonene will provide an initial scientific basis for whether or not there is benefit for including terpenoids in the development of cannabinoid-based pharmaceutical products as one means of reducing the incidence and/or severity of side effects.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

Protocol Overview. The proposed study will be conducted at the Johns Hopkins Behavioral Pharmacology Research Unit (BPRU). The purpose of the study is to examine the behavioral pharmacology of THC, d-limonene, and their combination versus placebo. All procedures will be performed in double-blind manner using a within-subject crossover design. Participants will be healthy adults with a history of having experienced mild to moderate anxiety following acute cannabis exposure, but will not be daily cannabis users. A total of 9 primary outpatient drug administration sessions will be conducted for each evaluable participant. Study completers who report increased anxiety after THC exposure will be invited to complete an optional 10th study condition with a higher dose of d-limonene.

1) Placebo (5mL distilled water)
2) THC (15 mg)
3) THC (30 mg)
4) D-Limonene (1 mg)

5) D-Limonene (5 mg)
6) THC (15 mg) + D-Limonene (1 mg)
7) THC (30 mg) + D-Limonene (1 mg)
8) THC (15 mg) + D-Limonene (5 mg)
9) THC (30 mg) + D-Limonene (5 mg)
10) THC (30 mg) + D-Limonene (15 mg)– optional for select study completers only

We will obtain a battery of pharmacodynamic outcome measures at baseline and for 6 hours after each dose. Sessions will be conducted at a target rate of 1-2 times per week, with a minimum of 3 days between experimental sessions. We will recruit study volunteers until 20 participants complete the protocol. Participants who drop out of the study prior to completion of all scheduled sessions will be considered "incomplete" and will be replaced. Drug administration sessions will be completed in a randomized order because there are too many dose conditions to properly counterbalance the order across 20 study completers. The optional 10^{th} condition will not be randomized and will only occur after the other nine study conditions have been completed and the PI has determined that the participant exhibited an anxiogenic response to THC during initial test sessions.

Participants. We will recruit and consent up to 65 research volunteers in order to obtain 20 study completers. We anticipate that about 50% of those screened will not be eligible or interested in the study, and that post-randomization some participants may drop out of the study before completion. It is estimated that we will need to randomize 32 participants to achieve 20 completers given the length of the protocol.

The target demographic for study participation are healthy adults who: have a history of intentionally consuming cannabis, self-report having experienced mild to moderate anxiety after using cannabis in the past, have not used cannabis more than an average of twice per week in the 3 months prior to study participation, and who are not currently dependent on or seeking treatment for use of cannabis or other drugs, including alcohol.

The selection of participants who have used cannabis, but are not current frequent users allows us to recruit individuals familiar with the effects of THC without issues of high levels of THC tolerance that might be present in daily users that could impact study outcomes. We will select individuals who self-report a history of mild-moderate anxiety after using cannabis in order to increase the likelihood of observing THC-induced anxiety in this sample; which is important for evaluating the primary study endpoint. Participants must report having inhaled, either via smoking or vaporization, 30mg of THC and 5mg of limonene at least once in their lives and not experienced excessive adverse effects. This will be assessed via an unstructured interview. Participant must report having inhaled 1 gram of cannabis (or an equivalent cannabis preparation believed to have delivered 30mg of THC and 5mg limonene in a single administration session. Any participant that reports having had an adverse reaction to cannabis that resulted in seeking medical treatment will be excluded.

Participant recruitment. Participants will be recruited into the study via media advertising (e.g. newspaper, internet) and word-of-mouth communication. Advertisements will seek healthy adults who occasionally use cannabis and are not currently trying to quit. Interested participants will receive an initial screening over the telephone, that includes interviews and self-report questionnaires that provide participant information regarding health status including physical, mental health, recreational drug use history, and experience of adverse effects following cannabis use, to determine eligibility for all criteria except those which require physical evaluation. Individuals who meet initial eligibility criteria will then be scheduled for an in-person physical evaluation.

Prior to the in-person assessment, written informed consent to participate in the study will be obtained. Urine specimens will be obtained and tested for evidence of recent use of commonly abused drugs. Participants must provide a government-issued photo ID confirming they are 18-55 years old, report prior use of cannabis, including at least 5 times in the past year, and report no allergies to cannabis or any of the test materials (e.g., lemon or similar citrus fragrances). Study participants will also undergo a physical

exam including clinical chemistry, hematology, serology, and serum pregnancy test (females only). Those who appear eligible for participation will receive training on the study assessment measures (e.g. exposure to subjective questionnaires and cognitive performance tasks), as well as instruction on using the Mighty Medic vaporizer. Participants who successfully complete training will be invited to participate in the study.

Experimental Session Procedures. For all study sessions, participants will be scheduled to arrive at approximately 8:00 in the morning on the day of cannabis exposure. Until such time as Covid-19-related restrictions on day-to-day operations are no longer required, social distancing will be maintained to the extent possible and all staff and study participants will be required to wear PPE throughout all face-to-face interactions. This will include mandatory use of face masks and, when closer than 6ft, use of face shields and disposable gloves. Participants will be provided PPE by study staff as necessary.

All participants will complete a breath alcohol test on arrival. Urine drug and pregnancy testing will then be conducted for all participants to test for evidence of recent illicit drug use (e.g. cannabis, cocaine, opioids) and pregnancy. Participants with a positive BAL or urine drug screen positive for any drug except THC will be sent home and the session re-scheduled. Participants who have a 2nd positive BAL, 2nd positive urine drug screen or confirmed positive pregnancy test will be immediately discharged from the study. The Time Line Follow Back (TLFB) procedure will be conducted to record substance use since the last study visit (intake assessment or prior experimental session). Concomitant medications**,** including vitamins and herbal supplements taken within 14 days prior to the first experimental session and throughout study participation will be recorded. Changes in medication occurring between the screening assessment and first experimental session, or between subsequent experimental sessions will be reviewed by a study investigator and medical staff prior to starting the session to ensure the volunteer is still eligible to participate.

Baseline Assessments. Prior to drug administration, the following baseline assessments will be completed: 5mL serum blood sample, vital signs (HR, BP), subjective drug effect questionnaire, and a brief cognitive performance battery (see below for details).

Experimental Drug Exposure.
Study drugs, pure THC in ethanol, pure d-limonene, or placebo (distilled water), will be delivered via vaporization using the Mighty Medic (Storz-Bickel, Tuttlingen, Germany), a commercial vaporizer designed specifically for the delivery of cannabis and THC. Vaporization was selected as the route of administration because THC and limonene have good pulmonary bioavailability, it allows for more precise dose delivery versus smoked or oral routes, this method is most likely to protect the blind of drug conditions between sessions as there are fewer sensory cues associated with inhalation of vapor versus smoked cannabis, inhalation is the most common method of consuming cannabis, and because vaporization has the same pharmacokinetics, but less pulmonary risk compared with smoking. The Mighty Medic employs hot air at a temperature of 210°C to vaporize THC and terpenoids without combustion, thereby limiting exposure to potentially carcinogenic polyaromatic hydrocarbons, ammonia, and other toxins. Each drug dose is placed in a small dosing capsule (or “pod”). Participants inhale the contents of the capsule by inhaling though a mouthpiece attached to the vaporizer. This activates the heating element and delivers the study drug. A new mouthpiece tip will be used for each experimental session, and a new vaporizer will be used for each study participant. The Mighty Medic is an approved medical device in the European Union, Canada and Israel and functions similarly to the Volcano Medic, which we have used previously in our laboratory to administer raw cannabis via vaporization. The Mighty Medic and Volcano Medic are made by the same company: Storz-Bickel. Analytical testing conducted by Research Triangle Institute and others has demonstrated that the Volcano Medic reliably and dose-dependently delivers THC and d-limonene. Importantly, analytical testing conducted by Stortz and Bickel has revealed that the Volcano Medic and Mighty Medic are equally effective at delivering THC in vapor. Currently, there are no regulatory limits in the U.S. on the allowable concentration of d-limonene in ambient air, and the total exposure during an acute dose session in this study is likely not more than what one would be exposed to peeling an orange or walking down an isle of cleaning supplies at a grocery store.

A pharmacist or other qualified technician will apply test substances with a micro-pipette into a dosing capsule, accessories that come with the Mighty Medic. For each experimental session, a dosing capsule with the assigned dose will be provided for participant self-administration using the Mighty Medic in accordance with the manufacturers operating instructions. When the Mighty Medic is activated, the dosing capsule is heated, vaporizing the substances placed inside it.

At each session, study personnel will first purge residual ethanol in the Mighty Medic in an unheated state. Detailed instructions for use of the device are available here: https://www.storz-bickel.com/media/wysiwyg/CRAFTY-MIGHTY/PDF/mighty-vaporizer-instructions-manual.pdf (see annex documents) and are provided in the supplementary materials section of the IRB application.

Participants will then inhale the contents of one Mighty Medic dosing capsule. Specifically, they will be given 15 minutes to inhale the contents of the capsule *ad libitum* (i.e., at their own pace). Participants will take a minimum of 15 puffs. If a visible vapor is still observed after 15 puffs, the will continue to take puffs until they no longer exhale visible vapor (this signifies that the dosing capsule is depleted; see detailed instructional materials from Stortz and Bickel). This procedure will produce THC exposure of 0, or 15mg or 30mg THC, and 1mg, 5mg, or 15mg d-limonene. Participants will be able to halt self-administration of the study drug if they experience adverse effects prior to inhaling the entire dose. Similarly, study staff may halt the drug administration procedure if untoward drug effects are observed. This is not expected at the doses under investigation, but may occur in rare circumstances. Study participants and research staff will be blinded to dose assignment. The conclusion of drug administration will be considered the “0 hour” by which remaining protocol assessments will be scheduled. To prevent issues of cross-contamination, new inhalation bags and thoroughly cleaned stainless steel wool pads will be utilized for every test session.

Post-Drug Administration Procedures.
Following the “0-hour” time point (last exhalation of study drug), participants will complete a battery of assessments that includes:

1) Serum specimen collection at 0min, 15 min, 60 min, 120 min, and 180 min.
2) Subjective drug effect ratings on computerized questionnaires and vital signs assessments at 0 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 300 min, and 360 min.
3) Subjective ratings of mood at 0, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 300 min, and 360 min.
4) Cognitive performance tests at 0, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 300 min, and 360 min.

Use of medication or tobacco products will not be allowed during the study sessions. Study participants who regularly use tobacco products will be provided a nicotine patch upon request.

Outpatient Discharge. Participants will be discharged after completing final assessments (approximately 6 hours post-exposure). In prior studies conducted in our laboratory, this timeline has been adequate for healthy adults to resolve any effects from acute doses of cannabis that are similar to those used in the present protocol. If a study participant indicates the desire to be discharged from the study early, BPRU medical staff will review the self-reported rating of “drug effect” on the most recent subjective drug effect assessment, performance on the cognitive test battery, and conduct a brief interview with the participant prior to discharge. At the end of each session, study staff will compare vital signs and cognitive performance assessments with baseline data and engage in face-to-face conversation with the participant to ensure that they are fit to leave the unit. Cognitive performance tasks obtained measure psychomotor ability, working memory, higher-order cognitive functioning and attention. If the participant is able to cognitively engage with staff, vital signs are within normative range (HR < 100bpm, SYS BP < 150mmHg, DIA BP < 90mmHg), and performance is not below 20% of baseline, the participant will be cleared to leave without further evaluation. If any of these parameters are not met, medical staff will assess the participant and a formal field sobriety test will be conducted. Note, that multiple members of the BPRU cannabis lab have received formal training on administering filed sobriety tests by a Maryland State Police Drug Recognition Expert (DRE). If the participant reports a drug effect or exhibits behavior indicative of impairment/intoxication, the participant will be asked to remain at the BPRU until the drug effect subsides and they can pass a field sobriety test. If vital signs are out of range, then medical staff will evaluate and

make a determination with regards to whether it is safe for participant to be discharged or remain under observation. Participants will not be allowed to drive home; instructions will be provided at the screening session regarding the need to make alternative transportation arrangements. If a participant fails to arrange a ride, taxi transportation home will be coordinated by study staff and provided free of charge.

For ease of scheduling, we will target conduct of sessions on a weekly basis. Because there are 9 total sessions (with option for 10), some participants may want to complete at an accelerated rate. A minimum of 48 hours will separate each study session and no more than 2 sessions will be completed in a calendar week. These timing parameters will allow for adequate elimination of study drugs between sessions and keeps the frequency of exposure to acceptable levels given that the study population will be infrequent cannabis users at the time of study participation.

Study Measures. A battery of measures will be used to assess participant characteristics and drug effects during the study.

Screening. Initial study screening will be completed over the telephone. During the phone screening assessment, staff will conduct assessments to collect background demographic data (age, gender, self-reported race and ethnicity, height, and weight) and to determine study eligibility (e.g. Medical History Interview, Drug-History Questionnaire, Time Line Follow Back (TLFB) assessment of all substance use for the prior 90 days, self-report of adverse effects following cannabis use). If the volunteer appears eligible based on this interview, a physical examination will scheduled to be completed at the BPRU by medical staff. All major organ systems, including head, eyes, ears, nose, and throat (HEENT); cardiovascular system; lungs; abdomen (liver/spleen); extremities; skin; central nervous system (CNS); musculoskeletal system, and general appearance will be assessed. Biological specimens will be collected and tested for routine clinical chemistry, hematology, serology, serum pregnancy test (females only), and for evidence of recent illicit drug use.

Experimental Sessions. Vital signs (heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP)) will be measured in the seated position using an automated monitor.

Six milliliters of blood will be collected by venipuncture or IV catheter insertion at baseline and again at the 0, 15, 60, 120, and 180-minute post-inhalation time points into vacutainer tubes. Blood will be spun to separate plasma, which will be labeled and stored frozen at -80 ºC until shipped frozen on dry ice to a designated laboratory for analysis. The maximum amount of blood to be collected is 36 mL per session, and 324 mL over the course of the entire study (those who complete the optional 10^{th} session will have 360 mL of blood collected total), which is less than the amount typically collected during a single routine blood donation (473ml). Quantitative levels of THC and its metabolites (e.g. 11-OH-THC and THCCOOH) will be obtained. Additional analytical testing (e.g. markers of limonene or stress) may be conducted as deemed appropriate to the current study.

A 22-item Drug Effect Questionnaire will be used to obtain subjective ratings of intoxication. Individual items include ratings of drug effects (i.e. drug effect, pleasant drug effect, unpleasant drug effect) and behavioral/mood states often associated with cannabis intoxication (i.e. relaxed, paranoid, hungry/have munchies). Participants will rate each item using a 100mm visual analog scale (VAS) anchored with “not at all” on one end and “extremely” on the other.

The 20-item State subscale of the State-Trait Anxiety Inventory (STAI) will be used to assess indexes of state anxiety/distress (e.g., current subjective feelings of apprehension, tension, nervousness, worry) before and after drug administration.

A brief battery of cognitive performance assessments will be conducted on aspects of functioning known to be sensitive to the acute effects of THC and cannabis, and which are relevant to functioning in the workplace and/or in operating a motor vehicle. All participants will be trained on the performance tasks to a stable baseline level during the screening session. Tasks include the Digit Symbol Substitution Task (DSST): Participants must hand type patterns presented to them on a computer screen for 90 seconds and outcomes include accuracy and total number of patterns completed in the allotted time; and a

computerized Paced Serial Addition Task (PSAT): Participants are provided a string of single digit numbers on the computer and must add the total of the prior to integers presented and respond by selecting the answer using the computer mouse on the screen, primary outcome is a summed score of the number of correct trials during the task. Recent studies in our laboratory have shown that these 2 tasks are sensitive to cannabis dose effects.

b. Study duration and number of study visits required of research participants.

Ten study visits will be required (11 for those who complete the additional 10th study session). One visit for screening evaluation, and 9 (with option for 10) outpatient experimental sessions lasting approximately 7 hours each. Because there are so many experimental testing sessions in this trial, and interpretation of data is contingent on a complete data set, participants may be invited to repeat a study session should circumstances arise that result in the loss of data during a session (e.g., failure to obtain blood specimens or missing cognitive testing time points), or protocol deviation that impacts data integrity. This is not expected to occur for most participants. Because the study involves repeated collection of blood samples, participants may not repeat more than 4 testing sessions (only 3 sessions may be repeated if they also completed the 10th session), as this would result in cumulative blood collection that would exceed what is typically collected during a single routine blood donation (473mL); see Risks, section 7, below. That said, it would be very unlikely for a participant to repeat more than 1 test session.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

THC/terpenoid dose assignment will be double-blinded in this study. That is standard procedure for appropriate scientific control in studies evaluating the dose effects of psychoactive drugs.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

e.

Participants in this study will be healthy volunteers. Routine care for any medical illness that may arise during participation will not be affected.

f. Justification for inclusion of a placebo or non-treatment group.

A placebo dosing session (no THC, no terpenoid) will be included to help interpret active drug effects on pharmacodynamic outcomes. Placebo dosing provides a control for expectancy effects on subjective reports and cognitive performance as well as non-pharmacological factors such as fatigue, hunger, and learning effects on performance tasks. Placebo dosing is standard for research studies involving evaluation of acute drug effects.

g. Definition of treatment failure or participant removal criteria.

This is not a treatment study. Participants may quit participation at any time of their own volition. The study investigators will discharge study participants for failing to attend their scheduled session, failure to follow the protocol requirements, or for other reasons not known at this time.

h. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

This is not a treatment trial; there is no direct course of therapy related to the participant population being targeted. We are recruiting healthy adults with experience using cannabis and who are not seeking treatment for substance use problems. Should any report the desire for treatment they will be referred to appropriate community service centers. Premature termination of participation may result in the need to recruit additional research volunteers, but should have no impact on the study volunteer directly.

5. Inclusion/Exclusion Criteria

Participants will meet the following eligibility criteria:

Inclusion Criteria

1. Have provided written informed consent
2. Be between the ages of 18 and 55
3. Be in good general health based on a physical examination, medical history, vital signs, 12-lead ECG and screening urine and blood tests
4. Test negative for drugs of abuse other than cannabis, including breath alcohol at the screening visit and at clinic admission
5. Not be pregnant or nursing (if female). All females must have a negative serum pregnancy test at the screening visit and a negative urine pregnancy test at clinic admission.
6. Have a body mass index (BMI) in the range of 18 to 36 kg/m2
7. Blood pressure at Screening Visit does not exceed a systolic blood pressure (SBP) of 150 mmHg or a diastolic blood pressure (DBP) of 90 mmHg
8. Have no allergies to any of the ingredients used to prepare vapor (THC, d-limonene).
9. Report having experienced anxiety after consuming cannabis in the past.

Exclusion Criteria

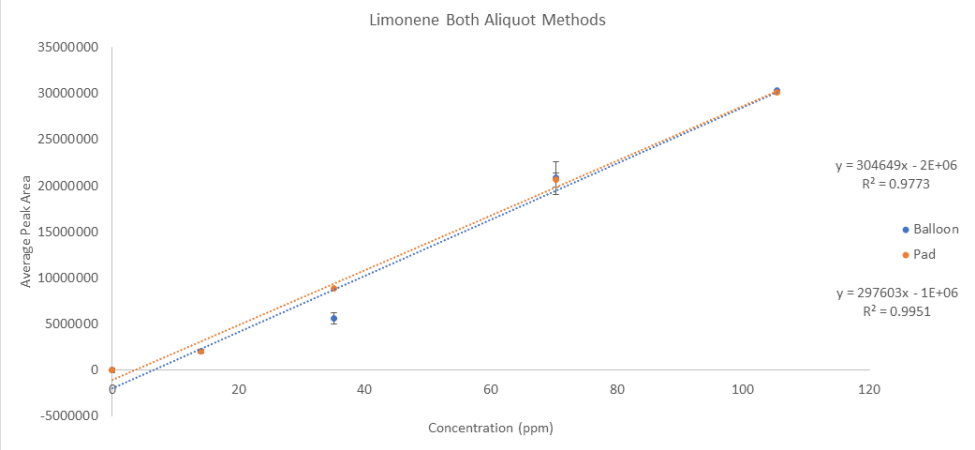
1. Non-medical use of psychoactive drugs other than, nicotine, alcohol, or caffeine 3 month prior to the Screening Visit;
2. History of or current evidence of significant medical (e.g. seizure disorder) or psychiatric illness (e.g. psychosis) judged by the investigator to put the participant at greater risk of experiencing an adverse event due to exposure or completion of other study procedures.
3. Use of an OTC, systemic or topical drug(s), herbal supplement(s), or vitamin(s) within 14 days or 5 half-lives of active drug (whichever is longest) prior to experimental sessions; which, in the opinion of the investigator or sponsor, will interfere with the study result or the safety of the subject.
4. Use of a prescription medication (with the exception of birth control prescriptions) within 14 days of experimental sessions; which, in the opinion of the investigator or sponsor, will interfere with the study result or the safety of the subject.
5. Use of dronabinol (Marinol®) within the past month.
6. Average use of cannabis more than 2 times per week in the prior 3 months.
7. History of clinically significant cardiac arrhythmias or vasospastic disease (e.g., Prinzmetal's angina).
8. Enrolled in another clinical trial or have received any drug as part of a research study within 30 days prior to dosing.
9. Having previously sought medical attention to manage adverse effects following acute cannabis use.
10. Individuals with anemia or who have donated blood in the prior 30 days

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

The THC for this study will be of GMP quality and manufactured and distributed to JHU by THC Pharm GmbH (Frankfurt, Germany) in accordance with federal regulations. THC Pharm GmbH will supply pure THC in a resinous form. The THC will be suspended in pharmacy-grade ethanol (190 proof) to create a solution that is approximately 10% THC/ 90% ethanol (this will increase the ease and precision of dosing measurements for THC, given the small doses that will be used). The ethanol solution will be purchased from Spectrum Chemical (product code: ET108; see appendix materials for product specifications), and meets standards for use in drug preparations intended humans. Prior to pulmonary administration, the ethanol will be dissipated from the heating pad used for THC administration. The Mighty Medic is an approved medical device for administration of THC in Germany and Canada and can reliably deliver cannabinoids with similar effectiveness to the Volcano Medic device (made by the same manufacturer), which has been used to deliver THC in research studies elsewhere (including in our laboratory).

The d-limonene for this study is >99% purity, meets GMP specifications, and will be obtained from True Terpenes. Analytical testing conducted by Brian Thomas, PhD, at RTI International indicates that the Volcano Medic reliably delivers d-limonene in a linear dose-response manner, and that recovery of d-limonene from the Volcano Medic balloons via GC/MS has little variability and does not differ whether the terpene is injected directly into the balloon and equilibrated with laboratory air or vaporized at 210°C using the Volcano Medic as will be done in the current study (see below Figure for triplicate testing via both methods). Given that the Mighty Medic and Volcano Medic are demonstrated to deliver comparable levels of cannabinoids and given that we will use the same temperature settings (210°C) for the Mighty Medic to vaporize THC and limonene, these analytical test results for the Volcano Medic are generalizable to the Mighty Medic.



The selection of doses was conducted to balance the study aim, participant safety and tolerability based on previous experience, and ensuring that doses are ecologically valid. In our laboratory, tolerant daily cannabis users have safely self-administered up to 3 grams of smoked cannabis containing 10% THC (300mg THC) within one hour (protocol NA_00082269). More recently, acute oral, smoked and vaporized administration of 10mg and 25mg THC to infrequent cannabis users (as will be recruited here) resulted in dose-dependent drug effects (IRB00035394). At the 10mg dose, participants reported an increase in pleasant drug effects with few unpleasant side effects. At the 25mg dose, there was no change in ratings of pleasant drug effects, but unpleasant drug effects, including self-reported ratings of anxiety, were significantly increased. Because the aim of the present study is to evaluate whether d-limonene can attenuate THC-induced anxiety, the selected THC doses represent what are likely to a good range from which to evaluate these effects. The low THC dose (15 mg) is intermediate to the 10 mg (no anxiety) and 25 mg (mild to moderate anxiety) doses we used in our recent protocol and is expected to serve as a positive control dose, although low levels of anxiety may be observed. The 30 mg dose is expected to produce anxiety in most study participants, but not severe anxiety in the target study population.

If initial testing in this study indicates difficulty with dose tolerability, or a lack of anxiety at either dose, then we will revise the proposed doses accordingly. Adverse events beyond anxiety, sedation, or nausea (vomiting in rare instances) are unlikely given the relatively safe pharmacological profile of THC (partial agonist), which has no history of being directly associated with fatalities. In cases where a participant experiences panic and or paranoid reactions, research staff will engage the person in relaxation exercises and will suspend research procedures until the volunteer has regained comfort. These types of effects are typically of short duration and our staff is well practiced in helping manage these types of effects. In the case of an extreme adverse reaction, we will call 911 and participants will be taken to the Johns Hopkins Bayview ER for treatment.

The d-limonene doses employed in this study (1, 5, 15 mg) were derived based on previous experiments outlined in the background (cf. Falk et al., 1990; Falk-Filipsson et al., 1993) and the known ratios of terpenes and THC in cannabis currently being used in legal markets in the U.S. and Canada. We are not aware of any serious adverse events occurring after inhalation of d-limonene. It is commonly inhaled in ambient air due to exposure to citrus fruits, household cleaners or air fresheners, candles, or a variety of other products containing citrus essential oils.

The Mighty Medic is an approved medical device in the European Union, Canada and Israel for inhalation of THC or cannabis (see certificate in Annex documents). The rationale behind its use is to volatilize cannabinoids and cannabis terpenoids at a temperature below that which combusts the material, and produces polyaromatic hydrocarbons (Abrams et al. 2007, Hazekamp et al. 2006, Zuurman et al. 2008).

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

THC is FDA approved as an oral formulation (dronabinol; Marinol). D-limonene is Generally Recognized as Safe (GRAS) for oral consumption. As detailed above, we believe that the THC and d-limonene administration in this study will be safe for cannabis-experienced participants. The route of administration (vaporization) is common for cannabis self-administration and the doses of both THC and d-limonene are within the range of what would be expected in real-world cannabis use scenarios. We have submitted and IND application to the FDA for the conduct of this experiment.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

N/A

Study Statistics

a. Primary outcome variable.

The primary outcome variable for this study is self-reported rating of “Anxiety” on the DEQ.

b. Secondary outcome variables.

Secondary outcome variables include self-reported drug effect and mood ratings on additional items of the DEQ and STAI, vital signs, cognitive performance, and Cmax of THC and 11-OH-THC in plasma.

The sample size estimate for this study was based on previous work in this laboratory evaluating dose effects of acute drug administration using a within-subjects design. A meta-analysis was previously conducted comparing the statistical power of 13 drug effect assessments from six dose-effect studies, with 14 participants each, evaluating a range of abused drugs in our laboratory (Felch, Di Marino, and Griffiths 1996). The analysis showed that average effect size for primary measures (i.e. subjective drug effect ratings, staff ratings and behavioral/cognitive performance measures) ranged from approximately 0.87 to 1.0. Based on this estimate of effect size, the proposed sample size of 20 should be adequate to assess the expected effects and to differentiate low from high doses. This sample selection methodology has been consistent in our long history of studies investigating dose-effects comparisons of different drugs, which have demonstrated excellent external validity and have become the FDA recommended standard for human abuse liability assessment. Subjective drug effect and mood ratings, vital signs and cognitive performance outcomes will be assessed using multiple regression analyses appropriate for repeated measures testing based on the final characteristics of the data set (e.g. normal distribution, skewness, kurtosis).

c. Early stopping rules.

The study will be stopped if new information is learned that indicates a serious risk to study participants.

7. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

Potential risks of THC exposure include dizziness, change in blood pressure, red or irritated eyes, drowsiness, easy laughing, euphoria, rapid heart rate, orthostatic hypotension, dry mouth, jitters, headache, nausea, vomiting, increased appetite, perceptual difficulties, memory lapse, hallucinations, confusion, depression, paranoid reaction, depersonalization, and rash. An additional potential risk of THC and/or terpenoid inhalation is coughing. Available preclinical and clinical evidence indicates that d-limonene does not pose a mutagenic, carcinogenic, genotoxic, nephrotoxic, or teratogenic risk to humans. In a prior experiment involving pulmonary inhalation of d-limonene at air concentrations that approximate the level of exposure expected in the present study (Falk-Filipsson et al., 1993), d-limonene exposure was not associated with any discomfort in the eyes, nose, or throat, and no CNS-related symptoms (e.g., “headache,” “fatigue,” “sick,” “dizziness,” “difficulty breathing”) were different from placebo. Exposure to d-limonene with THC may modulate or accentuate certain effects, and there is always the possibility of paradoxical reactions. However, we feel that the risk of serious adverse events related to THC or d-limonene exposure in this study is minimal, inasmuch as participants are experienced cannabis users and the doses we are administering are within the range by which most participants are likely to have encountered these substances through naturalistic cannabis use.

Venous blood sampling may cause pain, tenderness, bruising, or bleeding at the needle puncture site. Some subjects may feel transient lightheadedness or dizziness, or lose consciousness (syncope), because of anxiety and vasovagal reaction.

A further risk is that participants may mistake the proposed studies as treatment or may delay treatment seeking in order to participate, although this is unlikely since we are targeting occasional users.

Breech of confidentiality about self-reported drug use and biological tests indicating recent drug use is also a risk.

Exposure to COVID-19 is a risk.

b. Steps taken to minimize the risks.

Participants are not a "vulnerable population" as defined by human subject protection guidelines; that is, they are not minors, pregnant women, under legal coercion or restriction, or mentally impaired. They are competent adults who provide their voluntary informed consent. Participants will be recruited via media advertisements and posters that clearly state the nature and intent of the study. The consent process will inform the participant in detail of the procedures, time involvement, compensation, risk, and treatment options other than participation in our study. Particular emphasis will be given to providing information regarding the potential risks involved with taking the study drugs. Volunteers will also be instructed that they may withdraw from participation at any time without losing any of the compensation that they have earned to that point.

It is unlikely that any adverse event should arise that requires immediate medical or psychiatric treatment. However, in case of an adverse event, participants will be under the supervision of medical/nursing staff throughout the study. The medical and nursing staff at BPRU are trained in CPR and mobile emergency crash carts are available on the same corridor where all experimental procedures will be conducted. The research facility (BPRU) is located directly across the street from the Johns Hopkins Bayview Medical Center Emergency Department, and, in case of an adverse event, staff will call 911 and participants will be taken by EMTs for immediate care. The Principal Investigator will be immediately notified of any serious adverse events that arise.

If participants develop nausea or vomiting after vaporization, study staff will assist the affected participant(s) appropriately and contact the study PI and BPRU medical staff. Nursing staff will be on site during all experimental test sessions and a physician is always on call.

Blood collection risk will be minimized by performing venipuncture while participants are sitting down, and by having them remain under staff observation until it is clear that no acute adverse effects occur as a

result of the procedure. The risk of infection is negligible because standard sterile technique will be used. Venipuncture poses a risk of infection or thrombophlebitis, which increases with duration of placement. This risk is minimized by use of careful sterile technique, having nursing staff check the venipuncture site (with prompt attention if there are clinically significant signs or symptoms such as tenderness, swelling, or redness). The risk of anemia is negligible because the total amount of blood to be collected (up to 360 mL) during the entire completed study is less than the amount (473 mL) collected within one hour during a single blood donation session. The amount of blood loss will be readily replaced without harm to study participants.

All advertisements and the informed consent process will clearly indicate that this research is designated only for those not seeking treatment, that participation is not a substitute for treatment, and that participation offers no clinical benefit. They will be clearly informed that they will be asked to inhale cannabis components during their participation. Any participant who expresses an interest in receiving immediate treatment for cannabis or other substance use will be referred to a community treatment clinic. If this occurs during the study, their participation in the study will be terminated. As previously described, participants will be instructed that should they withdraw from the study at any point to pursue treatment they will still be compensated for their participation up until that point in the study.

Until risk of exposure to COVID-19 is no longer a public health concern, we will maintain social distancing throughout the study procedures to the extent possible. All staff and study participants will be required to wear PPE appropriate to the nature of the tasks being completed and distance to others (e.g. face masks when maintaining social distancing, face masks, face shields, and disposable gloves when closer than 6ft). We will also minimize the number of staff who come in contact with any single participant to the extent possible based on duties to be performed and staff availability. Our target will be to have each participant only interact with 3 staff (medical staff member for physical evaluation, nurse for blood draws, research staff member for all other procedures).

c. Plan for reporting unanticipated problems or study deviations.

Study personnel will also follow ICH regulations (detailed in *Clinical Safety Data Management, Definitions, and Standards for Expedited Reporting)* regarding reporting of adverse events and all study deviations to the IRB and study sponsor.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Participants' names will be recorded only on the screening, informed consent, and necessary medical and payment forms. Anonymous participant identification numbers will be used on all other forms and labeling of biological fluids and test results. All information gathered will be kept in locked research staff offices or file cabinets. All medical information obtained will be handled in accordance with HIPAA regulations. Only research staff will have access to participant records. The limits of confidentiality (e.g. suspected child abuse or neglect, or harm to self or others) will be discussed in detail with the participants during the informed consent process. To reduce the likelihood of patient records disclosure we have obtained a Certificate of Confidentiality.

e. Financial risks to the participants.

This study does not involve patients receiving treatment; therefore, the financial risks are minimal. Participants will be fairly compensated for their time and effort in complying with the study protocol.

8. Benefits

a. Description of the probable benefits for the participant and for society.

The primary benefit of the proposed research is in the knowledge gained regarding the pulmonary inhalation of THC and d-limonene alone and in combination. This knowledge will advance our basic scientific understanding of both substances and may guide policy and regulations related to cannabis. The study will also extend the extant literature investigating the acute dose effects of

inhaled THC, including subjective effects, cognitive performance, and their correlation with biological cannabinoid levels. These experiments may objectively demonstrate synergy of cannabis components and the modulating and even beneficial effects of cannabis terpenoids on THC. The results could benefit the research community in finding and developing selected cannabis chemovars with unusual cannabinoid and terpenoid contents and ratios for improved efficacy and optimized therapeutic index. Because we anticipate relatively minor risks to these cannabis experienced study participants, we feel that the proposed research has a positive risk-benefit ratio.

9. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

All participants will be compensated $30 for completing the screening assessment, up to $2700 for completing each of 9 outpatient sessions, and $500 in completion bonuses resulting in $3230 of total possible earnings for completing the entire study. Those who complete the optional 10th study session will be paid an additional $300, thus earning a total of $3530. Compensation of this magnitude is appropriate given the length and nature of this study. Calculations are as follows:

Screening Visit:	$30
Outpatient Sessions 1-9:	$300/day ($2700 total)
Completion Bonus:	$500
Optional 10th study session:	$300
Total Compensation for 9 main visits:	$3230
Total Compensation for 9 main visits +optional 10th visit:	$3530

Study participants will receive an additional $300 for any test session that is repeated due to data loss. There will not be any change to the study completion bonus for repeat testing.

10. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The only direct costs to the participants will be their transportation to and from Bayview for each study visit. That cost has been factored into the compensation for participating.

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