

1. PrCEPT Study Protocol and Analysis Plan

Funding: P01 DA032507-06A1 Pharmacology of Drugs of Abuse in Pregnancy

Title: Effects of Pregnancy-associated Hormones on THC Metabolism in Women

NCT number: NCT04374773

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2. Summary of the Protocol

Description of the protocol, procedures and table of events:

The clinical study will be conducted to investigate the induction of cannabis metabolism by estradiol and glucocorticoid (hydrocortisone) hormone treatment (Table 1). We have chosen to use hydrocortisone to mimic endogenous cortisol increase as observed in pregnancy instead of synthetic corticosteroid, as hydrocortisone is the most commonly used corticosteroid for physiologic replacement in clinical practice. We intend to study THC metabolism in enrolled subjects following administration of dronabinol once before (study visits 2 and 6; pre-hormone treatment control day; please see Table of Study Events below) and once after sequential estradiol and cortisol treatment (study visits 4 and 8; post-hormone treatment). During each visit, investigators will collect serial blood and urine samples to measure THC, THC metabolite, and hormone concentrations. Additionally, biomarker-based assessment of THC pharmacologic effects will be accomplished through measurement of heart rate and subject rating of side effects and psychoactive effects using a visual analog scale (VAS).

Procedures entail administration of medications, collection of blood and urine, buccal swab for genotyping analyses, and pharmacologic biomarker analysis. Subjects fulfilling eligibility criteria and providing informed consent at the screening visit (study visit 1) will arrive to the UW ITHS Adult Translational Research Unit (TRU) for each of the 4 study visits: twice before (study visits 2 and 6; pre-hormone treatment control days) and twice after sequential treatment with estradiol and cortisol (study visits 4 and 8; post-hormone treatment days). A baseline blood draw (time 0 hr) will be performed (24 mL of blood) to establish baseline levels of cortisol and estradiol and collect peripheral blood mononuclear cells (PBMCs). At the pre-treatment pharmacokinetic study visits, subjects will take a single dose of dronabinol (2.5 mg PO). The subjects will then collect their urine for 24 hours. Additionally, after placement of an intravenous catheter, venous blood samples (2-4 mL per time point) will be collected at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after THC dosing. The total volume of blood drawn will not exceed 75 mL. At each time point, pharmacological biomarker analysis will also be performed. Heart rate changes will be recorded as an objective measure of pharmacologic effects of dronabinol. At each time point, participants will also complete a questionnaire describing subjective effects related to mood, drug effect, and physical symptoms using a visual analog scale (VAS). After a minimum washout period of one week, the subject will have an estradiol patch applied for one week, delivering 0.3 mg/day or take hydrocortisone twice daily by mouth (30 mg/day; 20 mg in the morning and 10 mg in the afternoon) for one week prior to returning to the clinic for the post-treatment pharmacokinetic study visits. Dronabinol dosing, urine and blood sampling, and pharmacological analysis will be identical to that performed on the first study visit. Subjects will leave the TRU after the 12-hour timepoint for each pharmacokinetic study and present to the Center for Research in Reproduction and

Contraception (CRRC) for a study visit the following morning (study visits 3, 5, 7, and 9) to return the 24-hour urine collection and undergo blood sampling at the 24-hour timepoint.

Participants will be randomized for drug treatment order (e.g. whether they will undergo treatment with estradiol or hydrocortisone first). All study participants will undergo both drug treatments.

Table of Study Events

Procedure	Screening	Pre-treatment PK study, day 1	Pre-treatment PK study, day 2	Post-treatment PK study, day 1	Post-treatment PK study, day 2
Room #	Visit 1 CRRC	Visits 2, 6* ITHS Adult TRU	Visits 3, 7* CRRC	Visits 4, 8* ITHS Adult CRU	Visits 5, 9* CRRC
		Baseline: prior to estradiol or hydrocortisone treatment		After 1 week of estradiol or hydrocortisone treatment	
Consent/HIPAA	X				
Medical History	X				
Body weight and vital signs	X	X		X	
Physical exam	X				
Buccal swab for DNA testing	X				
Dronabinol administration		X		X	
IV catheter placement and blood draws over 12 hours		X		X	
Non-fasting blood draw	X		X		X
Urine pregnancy test	X	X		X	
Begin 24-hour urine collection		X		X	
Drug dispensing** (estradiol or hydrocortisone)			X		
Adverse event query		X	X	X	X
Payment	\$50	\$200		\$200	
<p>*Parallel visits (6, 7, 8, and 9) will occur after the second drug treatment period.</p> <p>**Participants will complete 7 days of treatment with either estradiol or hydrocortisone immediately following Visit 3 and Visit 7.</p> <p>PK = pharmacokinetic</p> <p>Pre-treatment PK visits will be scheduled on menstrual cycle days 0-3 for hydrocortisone and 15-17 for estradiol</p> <p>An additional \$150 will be paid upon study completion for a total compensation of \$1,000</p>					

Primary and Secondary Objectives and outcome measures

The goal of this aim is to test *in vivo* in women the hypothesis that increased concentrations of estradiol and/or cortisol lead to increased metabolic clearance of THC. The rationale is to specifically isolate the influence of pregnancy hormones in regulating CYP-mediated THC clearance in the absence of other complicating physiological changes that occur during pregnancy. This study is unique as, unlike studies done during pregnancy that demonstrate associations, **this study will test causality**. It will specifically test whether the pregnancy hormones commonly implicated in pregnancy-mediated regulation of CYP enzymes cause an induction of CYP activity *in vivo*. The obtained data will be **critical** for developing an understanding of how THC disposition varies between individuals and in predicting how the exposures to THC and its metabolites might be altered during pregnancy

The primary outcome measure of the study will be the change in the area under the plasma concentration-time curve (AUC) of THC following each of the interventions: exogenous estradiol and hydrocortisone exposures.

Estradiol phase:

Primary Outcome 1: Determination of whether estradiol alters THC exposure

The primary outcome is to determine whether the exposure to THC (as determined by AUC) is altered consequent to estradiol treatment when compared to the pre-treatment control and to quantify the magnitude of the change.

Secondary Outcome 1: Determination of whether other pharmacokinetic parameters of THC and its metabolites are different as a consequence of estradiol treatment

We will determine the time to peak concentration of THC, the peak concentration of THC, and the half-life of THC before and after estradiol treatment. We will also determine the AUC of metabolites of THC (11-OH-THC, 11-nor-COOH-THC, the glucuronides of 11-OH-THC and 11-nor-COOH-THC) before and after estradiol treatment. We will calculate the fraction of THC dose excreted into urine in the form of each of the metabolites and the renal clearance of each of the compounds based on urine and plasma data.

Secondary Outcome 2: Determination of the magnitude of effect of THC on heart rate in the presence and absence of estradiol treatment

As an objective measure of the pharmacologic effects of dronabinol, subjects will undergo monitoring of heart rate. The change in heart rate will be reported for each time point after dosing in comparison to baseline. The time course of change in heart rate will be reported and the change in heart rate at each time point after baseline will be compared to baseline. These data will be used as a descriptive measure of the magnitude and duration of the pharmacological effects of THC and to explore whether the doses used are pharmacologically active.

Secondary Outcome 3: Description of the side effects and psychoactive effects caused by THC in the presence and absence of estradiol treatment

Subjects will be asked to rate subjective effects related to mood, drug effect, and physical symptoms using a VAS as a function of time after dronabinol dosing. Subjects will complete VAS ratings at each of the blood sampling timepoints during the pharmacokinetic study visits.

Hydrocortisone phase:

Primary Outcome 1: Determination of whether hydrocortisone alters THC exposure

The primary outcome is to determine whether exposure to THC (as determined by AUC) is altered consequent to hydrocortisone treatment when compared to the pre-treatment control and to quantify the magnitude of the change.

Secondary Outcome 1: Determination of whether other pharmacokinetic parameters of THC and its metabolites are different as a consequence of hydrocortisone treatment

We will determine the time to peak concentration of THC, the peak concentration of THC, and the half-life of THC before and after estradiol treatment. We will also determine the AUC of metabolites of THC (11-OH-THC, 11-nor-COOH-THC, the glucuronides of 11-OH-THC and 11-nor-COOH-THC) before and after hydrocortisone treatment. We will calculate the fraction of THC dose excreted into urine in the form of each of the metabolites and the renal clearance of each of the compounds based on urine and plasma data.

Secondary Outcome 2: Determination of the magnitude of effect of THC on heart rate in the presence and absence of hydrocortisone treatment

As an objective measure of the pharmacologic effects of dronabinol, subjects will undergo monitoring of heart rate. The change in heart rate will be reported for each time point after dosing in comparison to baseline. The time course of change in heart rate will be reported and the change in heart rate at each time point after baseline will be compared to baseline. These data will be used as a descriptive measure of the magnitude and duration of the pharmacological effects of THC and to explore whether the doses used are pharmacologically active.

Secondary Outcome 3: Description of the psychoactive effects and side effects caused by THC in the presence and absence of hydrocortisone treatment

Subjects will be asked to rate subjective effects related to mood, drug effect, and physical symptoms using a VAS as a function of time after dronabinol dosing. Subjects will complete VAS ratings at each of the blood sampling timepoints during the pharmacokinetic study visits.

Inclusion and Exclusion Criteria:

Inclusion Criteria

- a. Healthy, premenopausal women ages 21-45 years old
- b. Normal menstrual cycles (monthly, cycles 28-35 days in length)
- c. Body mass index $<30 \text{ kg/m}^2$
- d. Must be willing to use nonhormonal methods to avoid conception during the study period
- e. Must be willing not to take any known inhibitors or inducers of CYP2C9, CYP3A4 or UGTs from 3 weeks prior to the start of each study through the last day of study. This list includes but is not restricted to some anticoagulants, anti-psychotics, antibiotics, antifungal agents, antidepressants, herbal medications and HIV agents.

Exclusion Criteria

- a. History of diabetes or significant cardiac, kidney ($\text{eGFR} < 60 \text{ mL/min/1.73m}^2$), gastrointestinal or liver disease
- b. History of blood clots or stroke
- c. Allergy to dronabinol, synthetic steroids, or any other chemically related drug or steroid
- d. Current or recent ingestion (<3 weeks) of any medication or herbal supplement known to be an inducer or inhibitor of CYP2C9, CYP3A4 or UGT. These include some anticoagulants, anti-psychotics, antibiotics, antifungal agents, antidepressants, anti-retroviral agents and herbal supplements (or other over-the-counter medications and supplements). Subjects who are taking any of these prescription drugs will not be asked to discontinue treatment but will be ineligible for

study participation. Subjects taking excluded over-the-counter medications and/or supplements will be given the option of discontinuing these for 1 month prior to study participation.

- e. Current pregnancy or lactation
- f. History of use of illicit drugs or smoking within the last year
- g. Any recreational or medicinal use of cannabis or other forms of THC within 3 months
- h. Current use of amphetamines, anticholinergic drugs or antidepressants
- i. History of seizure disorder or psychiatric illness (mania or schizophrenia; major depression within the past year or >2 episodes lifetime)
- j. Current use of live or live attenuated vaccines
- k. Personal or family (1st degree relative) history of breast or ovarian cancer
- l. Systemic disease (cancer, auto-immune disease, chronic infection, etc)
- m. Current or recent (within 6 months) use of hormonal contraceptives
- n. History of severe hypertriglyceridemia (>300 mg/dL or history of acute pancreatitis)
- o. Uncontrolled hypertension (BP>140/90)
- p. Allergy to sesame oil
- q. Anemia (Hct <34 g/dL)
- r. Extensive skin disease (eczema, psoriasis, etc) that would preclude use of transdermal estradiol

Sample size and power calculation

We expect a 50% change in THC clearance to be clinically significant and, based on existing data on CYP2C9 induction by glucocorticoids and estrogens, we predict the clearance of THC to be induced by 50% in our study by one or both of the hormonal exposures. Hence, we have powered our study to detect a 25% decrease in the AUC of THC. Based on the published variability in THC and its metabolite disposition and the variability in the magnitude of drug interactions measured, a paired power calculation for 80% power with an α (Type I error rate) of 5% results a **target number of subjects of 12 for each DDI study phase**. We intend to consent and enroll 16 subjects to undergo both study phases to ensure 12 subjects who complete all study procedures and have a wild type genotype for UGT1A1 and CYP2C9.

As a secondary outcome, we will determine the effects of estradiol and hydrocortisone treatment on the AUCs of 11-OH-THC, 11-nor-COOH-THC and the 11-nor-COOH-THC-glucuronide. Of these, 11-OH-THC is an active metabolite, and it has also been shown to be much more greatly affected by rifampicin induction than THC. Based on the published variability in the exposures to the THC metabolites, 12 subjects will also provide us with adequate power to determine whether the exposures to these metabolites are altered by estradiol or hydrocortisone. The changes in AUC will be tested using analysis of variance on log-transformed data. Point estimates and 90% confidence intervals will be defined for treatment versus control in THC disposition in accordance to the guidance on statistical analysis of DDI studies. To establish the difference between treatment and control, % confidence interval of the geometric means must be outside of the range of 0.80 to 1.25. If any of our subjects have a CYP2C9*2,*3 and UGT1A1*28 genetic polymorphism, we will also analyze the data for subjects with the specific genotype separately to avoid variability introduced by genetic polymorphisms.

3. Trial Management

The procedures will be conducted within the UW Institute of Translational Health Sciences (ITHS) Adult Translational Research Unit (TRU) and the UW Center for Research in Reproduction and

Contraception (CRRC), both of which are housed within the University of Washington Medical Center (hospital). There will be no other clinics or data collection centers involved.

Table 2. Planned Enrollment table. We anticipate initiation of participant recruitment and enrollment roughly midway through Year 1, and we plan to complete enrollment during Year 4. All subjects will undergo sequential treatment with estradiol and hydrocortisone, with pre- and post-treatment doses of dronabinol for each hormone exposure. Subjects will be randomized to treatment order.

Grant year	Number of subjects enrolled during the year	Cumulative number of subjects enrolled
Year 1	3	3
Year 2	4	7
Year 3	5	12
Year 4	4	16
Year 5	0	16

Healthy, 21-45-year-old premenopausal, non-pregnant female volunteers will be enrolled to complete the study. Since the research is focused specifically on THC exposure during pregnancy, only women will be enrolled in the study. In addition, estradiol is approved for use only in women. Dronabinol is only approved for use in adults. Therefore, we will not recruit individuals under the age of 21. At present the psychological and developmental risks of dronabinol use in children are unknown.

We ask study subjects to volunteer information about their race and ethnicity, but this information is not required for participation in our studies. Our previous clinical research studies have had the following demographic distribution: 85% of our study subjects are Caucasian, 5% are African-American, 7.5% are Asian and 1% each Native American and Hispanic. This breakdown closely reflects the demographics of our community, and we remain committed to increasing enrollment of underrepresented minorities in our studies.

4. Data Management

Statistical analysis plan

The changes in AUC of THC after treatment with either estradiol or hydrocortisone will be tested using paired analysis of variance on log-transformed data with each subject serving as her own control. Point estimates and 90% confidence intervals will be defined for treatment versus control in THC disposition in accordance to the guidance on statistical analysis of DDI studies. To establish a difference between treatment and control, % confidence interval of the geometric means must be outside of the range of 0.80 to 1.25. This statistical analysis is concordant with the FDA guidance on analysis of drug-drug interaction data.

The changes in AUCs and formation clearances of metabolite parameters (the AUCs of the metabolites, and the formation clearances of each metabolite) will be tested using paired analysis of variance on log-transformed data with each subject serving as her own control.

The change in heart rate will be reported for each time point after dronabinol dosing in comparison to baseline. The time course of change in heart rate will be reported and the change in heart rate at each time point after baseline will be compared to baseline by repeated-measures ANOVA.

We will report the nature of observed psychoactive effects and the mean absolute VAS score for all the subjects at the specific time points in the two different study sessions (pre- and post-hormone treatment). The change in each subjective measure will also be reported for each time point after dosing in comparison to baseline. The time course of change in each subjective measure will be reported, and the change in each subjective measure at each time point after baseline will be compared to baseline by repeated-measures ANOVA.

5. QA and QC plan

Blood and urine samples and buccal swabs will be obtained directly from study participants. The samples will be labeled with a unique sample code that is linked to the donor, the type of specimen and the sampling date. Coded sample records are kept in a password protected computer and backed up on an independent computer server. We will obtain the sample code, collection date and time, drug administration records and sample volume data for each subject.

Valid QC procedures for authentication of the chemicals used in quantitative analysis and for quality assurance will be employed for all assays used for the proposed studies.

All bioanalytical methods will be validated according to the FDA guidance on bioanalytical method validation. For each analytical batch, independent standard curves and quality control (QC) samples are included. The QC samples are included at the concentrations of lower limit of quantification (LLOQ), mid-range concentration and at a high concentration reflecting the highest concentration among the samples analyzed. A minimum of three QC samples at each concentration are included in each bioanalytical batch. These QC samples are independently prepared and controlled to assure day-to-day reproducibility and accuracy of quantification. For quality assurance, instrument performance is independently monitored using standard reference materials to confirm that sensitivity and system noise are within 20% of the day-to-day average.

The collected experimental data will be independently analyzed by two individuals of whom at least one is blinded to the treatments used during analysis. In case the two individuals reach discrepant results, a third scientist trained in the discipline will repeat the analysis and conduct an independent data verification to assess sources of discrepancy. No data points, experiments or individuals will be omitted from the analysis based on subjective assessment. Potential outliers will be evaluated by an independent expert in biostatistics who will determine whether an experimental observation is an outlier. In any situation where one of triplicate observations appears to be an outlier, an additional experiment will be conducted to allow determination of potential outliers.

Sex will not be assessed as a biological variable in this study as the focus of the proposal is pregnancy mediated changes in THC metabolism.

6. Regulatory

All AEs, regardless of whether they appear related to study participation, will be recorded and reported to the IRB annually.

AEs that are Grade 3 or higher (serious AEs) will be reported to the IRB and the Data Safety Monitor (DSM) within 24 hours of learning of the serious AE (SAE) and to NIDA within 5 working

days. Any actions taken by the IRB due to SAEs or AEs will be reported to NIDA within 24 hours of the notice of the IRB action.

Any changes to this protocol will be reported to the NIDA PO within 5 business days of IRB approval. No changes to this protocol will be implemented, and no study procedure involving any study subjects will be performed without approval by the NIDA PO of the modified protocol.

7. Trial Safety

Potential risks and benefits

Potential Benefits:

Study subjects will not receive any direct benefit from participating in the study. Participation in this study could provide extensive benefit to others, as findings may ultimately predict the disposition of THC in a vulnerable population - pregnant women - and aid in safety assessment of cannabis use during pregnancy.

Cannabis (marijuana) is the most commonly used recreational drug in the United States, and its use has become more prevalent in recent years. Women's health providers are increasingly likely to encounter women of childbearing age and women who are pregnant that use or have used marijuana. There is inadequate knowledge of marijuana's effects on pregnant women and their fetuses and inadequate screening for substance use during pregnancy. This study attempts to predict disposition of the active component of cannabis, THC, in pregnancy by supplementing subjects with estradiol and hydrocortisone to mimic hormonal changes observed during pregnancy and potentially altering THC disposition.

Potential Risks:

Physical Risks: Blood Sampling: Venipuncture may cause mild temporary discomfort, bruising, or bleeding at the needle insertion site. Although local infection is possible, this risk is very small, as venipuncture is performed only by experienced phlebotomists after alcohol cleansing of the site, and infections are not expected. Subjects occasionally experience lightheadedness or syncope with blood draws.

Physical Risks: Buccal Swab: The buccal swab may cause mild, temporary discomfort. No physical risks are associated with this procedure.

Physical Risks: Side effects of Study Medication: Subjects may experience side effects from the medications administered as outlined below.

Dronabinol oral capsule is commonly administered to patients with loss of appetite. The recommended dose of dronabinol oral capsule is 2.5 mg twice daily. The dose may be reduced to 2.5 mg once daily for patients who are unable to tolerate higher doses. The dose may also be increased gradually based on response and tolerability. Treatment in this study is short-term (single dose). Short-term adverse events that can occur at a higher incidence than placebo include confusion, increased appetite, nausea and asthenia. Risks of these side effects are minimized by using the lowest clinically approved dose which adheres to the recommended dosage form: 2.5 mg once daily.

Estradiol patches are mainly used clinically in postmenopausal women with a typical replacement dose of 0.025-0.1 mg/24 hr. We expect transdermal estradiol at the administered dose (0.3 mg/24 hr) to increase circulating estradiol levels by ~200-280 pg/mL. Luteal phase estradiol levels can vary widely, from about 50-200 pg/mL. Thus, we anticipate that treatment will at least double estradiol exposure in most participants to ~250-480 pg/mL. This change in estradiol is the minimum needed to model the effects of estradiol on THC metabolism during pregnancy, when estradiol levels may reach 5,000-7,000 pg/mL. The risks of exogenous estrogens include increased risk of blood clots, elevations in blood pressure and plasma triglycerides, and increased risk of breast cancer and stroke in older women. Transdermal estradiol is the safest form of exogenous estradiol, and the risks of week-long dosing in pre-menopausal women are minimal. Nevertheless, as a conservative approach, we will exclude potential subjects with a history of blood clots, uncontrolled hypertension, moderate-severe hypertriglyceridemia, or a personal or family history of breast or ovarian cancer in a first degree relative. Short-term adverse events that can occur at a higher incidence than placebo include rashes, edema and elevated triglycerides. The subjects may also experience gastrointestinal changes and irritation, mood and sleep changes, headaches, hypertension, application site reaction, nausea, breast tenderness/swelling, changes in menstrual flow or cycle and leg cramps.

Hydrocortisone oral tablets are commonly used in the treatment of adrenal insufficiency at physiologic replacement doses of 15-20 mg/day. Most serious metabolic and skeletal side effects of hydrocortisone dosing occur only with long term treatment (>3 months) at supraphysiologic doses (>20 mg/day). A week-long dosing at 30 mg/day is not expected to cause these side effects and is not sufficient to confer secondary adrenal insufficiency, which requires an exposure >3 weeks. The risk of side effects is minimized by using the lowest dose that will result in cortisol exposure significantly higher than baseline and approach endogenous cortisol exposure during pregnancy, when cortisol levels typically rise 2-4 fold above baseline. However, some subjects may experience side effects from short-term hydrocortisone treatment including mood or personality changes and emotional lability, agitation, insomnia, hyperglycemia, changes in heart rate, fluid retention, low blood potassium levels, changes in appetite, muscle weakness, hypertension, gastrointestinal changes, infection and increased serum transaminases (usually mild elevations and reversible on discontinuation).

Psychological Risks: Subjects may experience anxiety associated with the interviews or study procedures.

Social Risks: Subjects will be asked about their history of psychiatric illness as well as recent illicit drug use and may incur stigmatization if discovered by others.

Cultural Risks: Subjects will be asked about their history of psychiatric illness as well as recent illicit drug use and may incur stigmatization if discovered by others in their culture.

Financial risks: Subjects may miss work for the study and therefore experience lost wages. Subjects will be informed that participation is voluntary, and they may refuse to participate and may withdraw from the study at any time without penalty. Subjects will be told that, in the event of a physical injury as the direct result of study procedures, they will be cared for by a member of the investigating team at no cost, within the limits of the University of Washington compensation plan.

Legal risks: Subjects will be asked about recent illicit drug use, which could pose a legal risk if discovered.

Risks to Privacy or Confidentiality: As with all studies, there is a risk for loss of confidentiality. To protect privacy, all specimens and research records will be identified only by code numbers, with the records linking the code to subject identifiers kept in a separate locked file accessible only by study personnel. Identifying information (names, addresses, phone numbers, etc.) will be excluded from any computer records. All research data and specimens used in subject genotyping, quantification of drug levels and in data analysis are identified by the unique subject identification number. In order to assure the rights and privacy of participants, only demographic and health questions necessary for the study will be collected. Overall, we believe that the risks of this study are acceptable.

Risk Mitigation plan:

Subjects are recruited from several sources, including the UW college community and databases of UW research volunteers. Posters/flyers will be placed on local community bulletin boards. Subjects who have previously participated in studies and have indicated that they would be interested in future studies may also be contacted. Trained members of the research team will obtain informed consent from each participant by using the following 5-step process: 1) subjects will have the protocol briefly described to them by telephone after they contact the study coordinator; 2) subjects who continue to express interest in the study will be scheduled for a screening visit with study personnel; 3) the informed consent document will be reviewed at the screening visit in order to ensure enrollment of only fully informed subjects; 4) study personnel will answer any questions; and 5) the subject will sign the consent form, witnessed by study personnel. The original consent form will be maintained in the subject's file, in a secured location within the study PI's office. A copy of the consent form will be provided to the subject. Institutional Review Board (IRB) approval for all forms used and for the study as a whole will be obtained before initiation of the study. Individuals interested in the study will be advised that their participation in the study is voluntary and that they may withdraw from the study at any time without penalty.

All pharmacokinetic studies will be conducted in the ITHS Adult Translational Research Unit, which is housed within the University of Washington Medical Center (hospital), under the supervision of the study physicians (Dr. Amory and Dr. Rubinow), who are thoroughly trained and experienced in the administration of the study drugs, as well as assessment and management of adverse effects. Study visits conducted the following morning for the 24-hour time point blood sampling and return of the 24-hour urine collection will be held at the UW Center for Research in Reproduction and Contraception (CRRC), also housed at the University of Washington Medical Center and under the supervision of Dr. Amory and Dr. Rubinow. Subjects will also be continuously monitored by trained nursing (RN) personnel. Subjects will be monitored by noninvasive blood pressure and heart rate monitor and pulse oximetry and will receive supplemental oxygen if dictated, according to Good Clinical Practice. They are kept under observation in the ITHS Adult Translational Research Unit for 12 hours after dronabinol administration, which is longer than the expected duration of activity of the medication, so that by the time they leave the center, the medication should no longer be exerting any effects. Any participants who continue to complain of effects after this time will remain in the study center until the effects dissipate and they feel safe to go home. A taxi-cab service will be available to transport the participant home, and participants will be instructed that they will not be permitted to drive themselves home; they may arrange for a ride or utilize the taxi-cab service provided. Subjects will be provided contact information for study personnel, including emergency physician contact

numbers, so they may immediately report any adverse effects experienced during estradiol or hydrocortisone treatment.

Blood Sampling: Risks associated with venipuncture will be minimized by having blood draws performed only by trained personnel at the ITHS Adult Translational Research Unit and the CRRC. Only sterile materials will be used, and pressure will be applied to the skin after needle removal to minimize bleeding.

Buccal Swab: The buccal swab for genotyping will be performed by experienced study personnel. The procedure will be explained in advance to minimize any associated anxiety.

Side effects of Study Medication: To mitigate the chance of side effects, the lowest dose of dronabinol expected to have an effect will be used, and duration of treatment with estradiol and hydrocortisone is limited to week-long therapy. Participants whose medical history reveals contraindications for exposure to any of the study medications (i.e. history of blood clots pertinent to estradiol dosing) will not be able to participate in the study.

Psychological risks: Subjects may experience stress associated with interviews or study procedures, which may cause emotional stress or anxiety. Anxiety will be managed by maintenance of an open, supportive attitude by study personnel and careful explanation of study procedures. The procedures will be described to subjects prior to performance, and any questions or concerns will be addressed before the procedure is begun. Subjects are encouraged to ask questions and defer consent until all of their questions and concerns are addressed by the study personnel. Referrals to additional treating providers will also be made, if requested or clinically indicated (e.g. in the event that incidental findings from the screening tests are determined to be clinically significant, requiring further follow-up). Subjects will be further advised that they may withdraw consent at any time.

Social Risks, Cultural Risks: Subjects will be asked about their history of psychiatric illness as well as recent illicit drug use and may incur stigmatization if discovered by others. We will take extensive measures to protect the privacy of every participant (see below in confidentiality).

Financial Risks: Subjects may miss work for the study and therefore experience lost wages. We will work with the participants to plan for participation days that work best with their schedule. Participants will be compensated for their time during participation in these studies according to the predefined rate.

Legal Risks: Subjects will be asked about recent illicit drug use, which could pose a legal risk if discovered. We will take extensive measures to protect the privacy of every participant (see below in confidentiality).

Risks to Confidentiality: A very remote risk of breach of subject confidentiality exists, but extensive measures will be undertaken to protect the privacy of every participant. We create research charts that contain subject identifiers, source documents, laboratory test results, physical exams, and the signed consent forms. These charts are kept in locked cabinets in a locked office with limited access. Computer data files, biological specimens sent immediately for analysis, or those stored for future assay do not contain subject names — only subject codes. Any information sent to NIH, prepared for reports or manuscripts, or presented at scientific meetings will not contain the subjects' names. Social security numbers will be collected from subjects because they will receive monetary compensation for research participation. All research results are collected in a separate study chart stored in a separate location which only

includes the subjects' code and does not include any potentially identifiable information. Linkages to subjects are kept only on paper by the PI/study coordinator for the duration of the study and destroyed after data analysis is completed as required and compliant with Washington state law. All anonymized data will be stored on a secure, HIPAA compliant computer and will be password-protected. Access to the study data will require knowledge of the data format, file name, and computer password.

Trial Stopping rules

The Data Safety Monitor (DSM) will decide termination criteria for an individual subject and for the study as a whole based on the data. Specifically, any study volunteer can terminate her participation at any time without giving reasons. The Principal Investigator and/or study physician can discontinue a given participant if, in her/his opinion, the volunteer no longer meets the inclusion/exclusion criteria or clinical observations during the study suggest that it might be unsafe for the participant to continue.

Possible circumstances for study termination, "STOPPING RULES", for a particular participant or the study as a whole may include:

- Participant revokes her consent to participate in the study
- Medical prudence, at the discretion of the investigator
- Poor compliance or co-operation of the volunteer as judged by the investigator
- Use of medicinal products/drugs not prescribed by the investigator or not permitted during the study
- Serious adverse event(s)
- Non-tolerable adverse events in subjects
- Occurrence of a medical condition within the exclusion criteria
- Pathologically changed laboratory values in subjects
- Participant becomes pregnant

A final study visit may be conducted for study volunteers who prematurely terminate the study, or for whom the study is discontinued by the DSM. This visit may entail a physical exam and/or laboratory tests as needed to ensure the participant's safety. If the study is discontinued prematurely for any participant because of an SAE, the adverse event will be documented with copies of all associated evaluations and laboratory studies; the investigator must exclude the subject from the study immediately and report the event to the Institutional Review Board and the DSM.

Process of AE/SAE collections, assessing by PI and DSM

The investigators will closely monitor the clinical protocols described in this submission to ensure the safety of volunteers. Subjects will undergo vital sign monitoring throughout the pharmacokinetic study visits as well as pregnancy tests at the screening visit and prior to each administration of dronabinol. Participants will be queried about AEs at all study visits and provided emergency contact information. An AE will be defined as any symptom or abnormal laboratory value that develops or increases in severity during the course of the study and is determined to be clinically relevant.

AEs are recorded at all study visits on dedicated forms and characterized in terms of their start and stop date, maximum intensity, action taken on trial medication, and subject outcome and graded as follows:

Grading of Adverse Events (AEs): AEs will be classified according to severity as below.

Grade 1: A Grade 1 complication is any reported side effect that does not require pharmacologic treatment. An example may be nausea, change in appetite, mild diarrhea or mild infection such as an upper respiratory infection or a headache.

Grade 2: A Grade 2 complication is any that requires pharmacologic treatment. These include minor conditions requiring treatment (e.g. a headache requiring medication).

Grade 3: A Grade 3 complication is one that requires surgical, endoscopic, or radiologic intervention. Grade 3a indicates that the intervention does not require general anesthesia, whereas intervention for a Grade 3b complication requires general anesthesia.

Grade 4: A Grade 4 complication is any life-threatening complication that requires ICU level care. This may involve single organ dysfunction (e.g. dialysis, Grade 4a) or multi-organ dysfunction (Grade 4b).

Grade 5: Death of a patient.

Study investigators will monitor side effects and AEs at the study visits and the recording and reporting of all AEs will be as described above.

If a subject discontinues the trial because of an AE, this is also noted on the AE form. In the event of an SAE (grade 3 or higher), study investigators will complete and submit an Adverse Event Report form to our local IRB and the DSM (see below) within 24 hours of learning of the SAE. In addition, written summaries of the study status will be submitted to the local IRB and NIDA on a yearly basis, or more frequently if requested. Screening laboratory results are reviewed and signed by one of the study physicians on receipt from the clinical laboratory. Our clinical trial may be stopped if emerging effects demonstrate that the volunteers are at risk of SAEs from the study procedures.

8. Trial efficacy

Not Applicable. This study is not a Phase 3 efficacy trial and does not aim to assess efficacy or safety of a medication or device.

9. Administration of DSM Plan

The data and safety monitoring plan (DSMP) will be the responsibility of the study physicians Drs. Amory and Rubinow who will work closely with Dr. Nina Isoherranen, PhD (Study PI), to ensure completeness of the DSMP. These individuals will closely monitor the clinical protocols described to ensure the safety of volunteers. When an individual subject completes the study, Dr. Amory will review the AE reporting and the study record for that subject. All adverse events, study enrollment and any relevant findings will be reported to the local IRB annually.

A detailed DSM report will be submitted to NIDA annually that includes a description of study progress, enrollment updates, retention and disposition of participants, AE and SAE listings and any regulatory issues such as amendments, protocol deviations, IRB reports and QA issues.

An independent DSM, Dr. Mara Roth, who is without conflicts of interest with regards to the study or the study investigators, will perform biannual reviews of this study.