PROTOCOL AND STATISTICAL ANALYSIS PLAN

Study Title: **Sedative-Anxiolytic Effects on Simulated Driving Performance**

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Protocol

Background: Both the White House Office of Drug Control Policy and the National Institute on Drug Abuse have identified drugged driving as a research priority. A 2010 National Highway Traffic Safety Administration (NHTSA) study found that 18 percent of drivers killed in accidents tested positive for at least one drug. Specific benzodiazepines, drugs well-known for their impairing sedative properties, were unsurprisingly the first and fourth most common drugs identified in driver autopsies (12.1 percent alprazolam, 8.4 percent diazepam, respectively). It is unknown whether these people were using benzodiazepines as prescribed or had even obtained them legally. It is also unknown if the benzodiazepine contributed to the fatal accident. Zolpidem, a drug similar in many ways to alprazolam, recently underwent labeling changes due to adverse effects on driving ability the day after ingestion. Specifically, the FDA reduced the recommended starting doses of zolpidem-containing products (e.g. Ambien®, Intermezzo®) for women because average plasma levels of zolpidem were determined to be ~50 percent higher in women than men even after eight hours, meaning that women may have been unknowingly impaired during their morning commute.

Given the similarities in drug properties between alprazolam and zolpidem (both bind the benzodiazepine-binding site on $GABA_A$ receptors and both are metabolized by cytochrome P450 3A4), the unwanted carry-over effects may occur with both drugs. A clinical laboratory study by Norman, Burrows & McIntyre reported that 2mg alprazolam given at night adversely affected choice reaction time 11 hours later compared to placebo in healthy volunteers. Another measure of psychomotor impairment (critical flicker fusion) was also impaired in subjects 11 hours after 2mg alprazolam administration compared to their own baseline. A primary aim of the proposed studies is to investigate the effects of the most commonly prescribed benzodiazepine, alprazolam, on next-day driving ability- an outcome measure of great public health and safety significance.

Gender differences in drug response can be extensive, but too often these differences are not tested for during drug development and approval processes. Women participate in clinical trials about a third as often as men, meaning that we know less about how drugs will affect the half of the population that uses them the most. Indeed in one study of ~30 million prescription claims over the course of a year, men received fewer than four prescriptions while women received five (even after accounting for oral contraceptives). Gender differences in drug response can be due to disparities in pharmacokinetic processes (i.e. absorption, distribution, metabolism, excretion) between men and women. Women can have lower drug clearance due to slower glomerular filtration rate; the activity level of liver cytochrome P450s may differ between men and women; and volume of distribution for some drugs depends of percentage body fat, which is typically higher in women. Variations in any one of these factors from the mean can alter drug concentrations in the body, resulting in deviation from the normal range of effects. This may manifest in several ways, such as women experiencing more adverse drug effects; requiring different doses than men such as in the cases of anesthesia and some antipsychotics; or accidental drugged driving resulting in involvement in more accidents the morning after taking sleeping medication. A secondary objective of this study is to assess potential gender differences in next-day alprazolam effects. In summary, the similarities between zolpidem and alprazolam warrant further exploration of alprazolam effects the day after ingestion.

1. **Objectives:** The primary objective of this study is to assess the next-day effects of immediate-release alprazolam (.5, 1 & 2 mg) on driving impairment in healthy male and female volunteers using a driving simulator. Secondary objectives are to characterize the relationship between subject-rated impairment and driving simulator performance and to assess potential gender differences in hangover effects of alprazolam.

2. **Study Design:** This randomized, placebo-controlled study will consist of six overnight inpatient visits and will be conducted at the UK Center on Drug and Alcohol Research and the CCTS inpatient unit located in the UK hospital.

3. **Study Population:** Participants will be approximately 72 healthy adults (18 to 50 years old) who sign the screening consent with the aim of completing up to 24 volunteers. Written informed consent will be obtained prior to admission and the subjects will be paid for their participation. **Inclusion Criteria** include: 1) Male or female English-speaking literate adults, 18-50 years old, 2) valid driver's license, 3) non-regular nicotine users (includes e-cigarettes, nicotine replacement therapy, etc.). **Exclusion criteria** include: 1) benzodiazepine use within last 30 days, 2) lifetime history of benzodiazepine dependence, 3) seeking treatment for drug or alcohol use, 4) pregnant or lactating, or planning to become pregnant during study, 5) significant ongoing medical problem (e.g. diabetes), 6) serious psychiatric illness, 7) recent use of agents that are strong

inhibitors or inducers of cytochrome P450 3A4 (CYP 3A4), such as some azole antifungals (e.g., ketoconazole), macrolide antibiotics (e.g., clarithromycin), or protease inhibitors (e.g., ritonavir, indinavir, and saquinavir), 8) a known hypersensitivity to the study drugs, 9) intolerance of the simulated driving procedure (i.e. simulator sickness), 10) acute narrow angle glaucoma

We expect to begin recruiting by May 2016 and hope to finish recruitment by April 2018 with data analyses proceeding thereafter. We anticipate screening approximately three subjects for each one enrolled; thus, we anticipate screening approximately 72 volunteers to obtain up to 24 who complete the study (these numbers allow also for some attrition as we know that there will be individuals who are dismissed or drop out before completion).

5. **Subject Recruitment Methods and Privacy**: Volunteers will be recruited through newspaper and radio advertisement, local postings, online forums (e.g. ResearchMatch), and word-of-mouth. Volunteers will make initial contact by phone with one of our staff who have completed the research training and HIPAA compliance web-based teaching modules. We have obtained a waiver for our phone screen procedure, which is approved for our current studies (e.g., #11-0100-F6A). If the volunteer discloses information that would make him/her potentially eligible for the study, they will be invited to come in for a screening appointment. Screening is completed by one of our trained research assistants/research nurses/investigators at the Center on Drug and Alcohol Research Building or the UK CCTS. Study investigators may interact with volunteers in any of these settings and appropriate cautions are in place to ensure privacy during the intake process. We will submit advertisements that can be used for recruiting this target population for review by both UK PR and the UK IRB before advertising.

6. **Informed Consent Process**: Prior to obtaining informed consent, each potential subject must pass a field sobriety test and have an expired breath alcohol content (BAC) of 0.00. Sharon Walsh, Ph.D. or her designated Co- Investigators will obtain consent for this project. There are two consent forms for this project: a Screening Consent and the main Study Consent. Designated staff may conduct and sign the informed Screening Consent with the volunteer. However, the volunteer will meet with the investigator or co-investigator on an outpatient basis prior to admission to review all experimental procedures and allow the volunteer ample time to ask questions regarding the protocol before signing the Study Consent form. There is no time limit on this process. The investigator will inform the volunteer that signing the consent form does not obligate them to participate. Each subject will receive a copy of his or her informed consent document.

7. **Research Procedures:**
   Screening and Admission: Subjects will answer questionnaires related to their drug use and health during screening. Participants will be asked to give breath and urine samples for drug testing while a staff member is in the room. They will also undergo medical examination that includes a physical exam, electrocardiogram (ECG), and blood and urine chemistries. The number of screening visits may vary depending on the subjects' availability and staff scheduling. This study will enroll only healthy adult participants. Female participants will be tested at screening, and prior to each session for pregnancy. Participants will also drive the simulator to ensure they do not have an adverse reaction to it.

   Practice Sessions: There will be one to three practice sessions lasting 2 – 4 hours each. The volunteers will be familiarized with the experimental tasks they will complete during session. The number of practice sessions will depend on the volunteer's competency with the experimental tasks. Once the volunteer is adequately trained, they will be scheduled to complete the experimental sessions.

   General Methods: There will be a total of 6 experimental sessions and each will require an overnight stay. **Part I** of these sessions will occur the evening the participant arrives at CDAR. Once cleared for the study, they will complete a scenario on the driving simulator. Once completed, the volunteer will be escorted to the inpatient unit where they will stay overnight. All food consumption must be completed at least 3 hours prior to nightly dosing. Volunteers will be monitored to ensure they stay awake until dosing. At ~11:30PM, nurses will administer one of the oral study medications and the participant will be told to immediately attempt to sleep. The next morning **Part II** of the session will occur. Participants will be woken ~7 hours after drug administration. They will be transported to the experimental session room in CDAR, where the remainder of their session will be conducted. Participants will receive a single active drug (alprazolam .5mg, 1 mg & 2 mg; placebo or zolpidem 10 mg) during each of these sessions, with the order randomized both within and between subjects. Part II of the experimental sessions will last ~5 hours.

Experimental Session Part I Methods: On session days, participants will report to CDAR in the early afternoon Upon arrival, they will undergo drug, alcohol, and pregnancy testing. If all are negative, they will complete a scenario on the driving simulator that will serve as their baseline measurement for the session. They will be then escorted to the CCTS inpatient unit where they will spend the night. Participants will receive a dinner. At ~11:30, a nurse will administer a blinded study medication (either alprazolam [.5, 1, 2mg], zolpidem 10mg, or placebo) and instruct the participant to sleep.

Experimental Session Part II Methods: Participants will be woken ~7 hours after drug administration. They will complete a self-assessment of their sleep quality the previous night. They will be transported to the experimental session room in CDAR, where the remainder of their session will be conducted. After arrival at CDAR, participants will be allowed to eat a standardized breakfast. They will receive another blinded study medication (alprazolam 1 mg or placebo) at ~8:30AM. Only one active dose will be administered in a single session (i.e. if the evening dose is active, the morning dose will always be placebo). Only one morning dose will be active (alprazolam 1 mg). Data will be collected for approximately 5 hours. Outcome measures are detailed below. After session is complete, participants be required to pass a field sobriety task and will be provided transportation home. Sessions will be separated by at least two washout days (e.g. minimum of 63 hours between doses). Both zolpidem and alprazolam have half-lives that allow for adequate washout during this time frame.

Outcome Measures: Outcome measures include physiological measures (e.g. heart rate, respiration rate), subject-rated questionnaires (e.g. Visual Analog Scales for drug effects, Adjectives Scales, Pharmacological Class Questionnaire, Next-Day Questionnaire), observer ratings, and psychomotor tasks (including, but not limited to simulated driving). We have developed an approximately 8-minute driving scenario that includes highway and city settings. Outcome variables were based on the International Council on Alcohol, Drugs & Traffic Safety's (ICADTS) consensus recommendations for behavioral outcomes in drugged driving studies. These variables include braking reaction time to obstacles and standard deviation of lane position (a measure of swerving). Sleep monitoring may occur through use of a wrist monitor (such as a FitBit) and this will track total time spent asleep and sleep quality. Participants will also have their body fat percentage measured by DXA scan during one of their sessions.

8. **Resources:** The study will take place at our laboratory and at the University of Kentucky CCTS inpatient unit. Screening procedures and experimental procedures will largely be performed in the CCTS, where patients will reside. There will be 24-hr nursing supervision of volunteers while in the hospital. Dr. Michelle Lofwall will be the primary medically responsible investigator and is an adult psychiatrist with ACLS certification who has worked extensively with individuals with substance use disorders both in clinical and research settings. Dr. Lon Hays will supervise the Psychiatry Attending schedule to monitor subjects daily while they are inpatients. Dr. Walsh will provide oversight for the study. Overall, the study team and resources described above are well equipped to protect the participants and successfully implement, carry out, and complete this study protocol.

9. **Potential Risks:** The primary risks for the subjects in this study are associated with drug administration. All doses have been previously shown to be well tolerated both in clinical and laboratory settings. Side effects of alprazolam include sedation, motor impairment, and anterograde amnesia. Side effects of zolpidem include sedation, weakness, lightheadedness, headache, and nausea. Medical and nursing staffs are always prepared for the unlikely occurrence of a serious adverse side effect.

   During the screening process, it is possible that subjects may feel uncomfortable answering personal questions about their health, psychiatric, and drug use histories. Also during screening, participants will have blood drawn via venipuncture, which may cause soreness, bruising, pain, infection, possible fainting, and/or bleeding. Using sterile procedures and well-trained staff minimizes these risks. There is the risk that someone other than the research staff may see a subject's Protected Health Information. It is possible that participants will experience discomfort after using the driving simulator. Simulator sickness symptoms include headache, eyestrain, nausea, dizziness, and fatigue. These symptoms are temporary, and subjects will be excluded during screening if they are unable to comfortably complete a driving scenario.

10. **Safety Precautions:** Subjects will be carefully screened (history and physical examination, routine labs including CBC, urinalysis, ECG, and psychiatric assessments) to exclude those with a risk of adverse events. Those at increased risk may have histories that include a personal or family history of seizure or head injury associated with more than a brief loss of consciousness, hypertension, psychosis, etc. During sessions, subjects remain under careful observation. Vital signs will be collected throughout the dosing period. Before

subjects are released after experimental sessions, trained research staff will administer a field sobriety test to assess ambulatory ability. They will also be given transportation home. Dr. Walsh has substantial experience testing psychoactive substances in human subjects. Female subjects will be given pregnancy tests before each session to ensure that we do not administer any potentially harmful agents to a pregnant woman. To protect confidentiality, all research subjects are identified by a subject identification code (Subject ID) consisting of their initials and sequentially assigned subject numbers on all forms and data files, and not by their names. Actual subject names and corresponding subject IDs are kept in a locked master file separate from the actual data collected during the study. All personal and experimental information is kept locked and is accessible only to key personnel involved in the research. Several preventative measures will be in place to reduce the incidence of simulator sickness. Correctly positioning the driver, ensuring display alignment, keeping the simulator room cool, and advising the participant to avoid sudden or quick head movements all serve to decrease the likelihood of subjects experiencing simulator sickness.

11. **Benefit vs. Risk:** There are no direct benefits to volunteers. There are potential indirect benefits to society including the knowledge regarding the potential effect of alprazolam on next-day driving performance. Volunteers will indirectly benefit from receiving a free medical evaluation and free meals. The amount of risk to which individual study volunteers are exposed to is low. Overall, the risk/benefit ratio appears favorable, and the conduct of this research seems well justified.

12. **Available Alternative Treatment(s):** This is not a treatment study.

13. **Research Materials, Records, and Privacy:** Sources of research material obtained from our volunteers during screening and study participation include: blood and urine specimens, expired breath samples for alcohol, ECG data, self reported information on physical and mental health, family history, drug and alcohol use history, demographic information, volunteer and study staff observation of drug effects, vital signs (e.g. temperature, blood pressure, heart rate), and other physiological measures. All this information is required to determine eligibility for the study, to ensure safety during the experimental sessions and for outcome measures. All research material will be obtained and discarded when necessary in a HIPAA complaint manner. All materials will be collected specifically for the proposed study by trained staff. The principal investigator and medical team will have access to private health information about volunteers so that study eligibility can be determined. All data with personal health information is kept in a locked file cabinet that is separate from a locked file cabinet with de-identified volunteer data. Prior medical records may be obtained with volunteer consent if there is any question about the volunteers' health history. Each participant will sign a form that details the HIPAA compliant manner in which research material is collected. If, in the course of obtaining ECG or laboratory results, we discover a finding that warrants medical follow-up (elevated liver function suggestive of Hepatitis C or uncontrolled hypertension) we would provide the volunteer a copy of those labs and refer them to their general practitioner.

14. **Confidentiality:** Identifying information will be stored in a separate locked file cabinet from all other data and codes that could link an individuals' Subject ID to their identity. Incidental materials containing subject identifiers will be shredded or incinerated. Electronic data will be stored on password-protected computers in password-protected files. Access to identifying information will only be available to key research personnel. Paper records will be locked and stored for a minimum of 7 years and destroyed through shredding or a professional destruction company.

15. **Payment**: Participants will be paid for each screening visit at the rate of $25/visit. Volunteers will be paid $25 for each practice session. Participants will receive a base pay of $100/ study visit regardless of whether or not they complete the study. They will receive a bonus of $50/study visit if they complete the entire study, but not in the case of early departure or dismissal. Additionally, we will offer an on-time completion bonus for each driving scenario of $5. The study team and others have successfully employed this compensation structure before. If a participant completes the entire study (6 visits), with on-time completion of all drives, the total maximum earnings will be about $1,080 (excluding screening and practice).

16. **Costs to Subjects**: There will be no cost to volunteers for participation in the current study.

17. **Data and Safety Monitoring:**

    Medical Safety Monitoring: Volunteers undergo a rigorous screening process to determine their eligibility and safety of their participation. In addition, our well-trained and vigilant research and medical staff, and the

carefully considered medication dosing and safety criteria all serve as precautionary measures to ensure the safety of volunteers. The principal investigator, Sharon Walsh, Ph.D. will be the primary person responsible for monitoring the safety of this project and will comply with all reporting requirements. Additionally our medical staff, including Dr. Lofwall and CCTS nursing staff, will conduct careful medical monitoring. Volunteers will have daily contact with a physician and CCTS nurses while they reside as inpatients. The safety monitoring of each volunteer is discussed on an ongoing basis among medical and scientific staff, including our study statistician, Paul Nuzzo. This process has been successful in protecting volunteers and the integrity of the scientific outcomes. Any minor adverse events will be discussed among all study staff, documented in the subjects' medical charts through progress notes and medical logs along with any intervention required (e.g. headache requiring Tylenol). Any severe adverse events, whether study related or not, will be reported to the UK IRB and the FDA within 24 hours or as required.

Data Safety Monitoring: Data are collected using a computerized data collection and management system. The data are stored on password-protected computers in password-protected files. Data files do not contain PHI. A computer file linking the unique number with the subjects' name will be kept on a stand-alone, password-protected computer available only to the study investigators. Data collected on paper forms that require manual entry will be entered by two separate staff members and compared to ensure accuracy. All paper copies of collected data will be stored in locked file cabinets separate from any identifying information.

18. **Subject Complaints:** Subjects may ask study personnel questions about the study procedures or make complaints at any time. All staff will be aware to contact Marion Coe or Drs. Walsh or Lofwall about any subject concern or complaint as it arises. Phone numbers for the PI and staff, as well as the Office of Research Integrity are included in the consent form. It is expected that providing a phone number and contact information for the PI may offer a safe, confidential and reliable channel for participants to express problems, concerns or questions and obtain study information.

19. **Research Involving Non-English Speaking Subjects or Subjects from a Foreign Culture:** This research does not involve non-English speaking subjects.

20. **HIV/AIDS Research:** This is not an HIV/AIDS study and HIV testing is not conducted.

21. **PI-Sponsored FDA-Regulated Research:** The investigator is not the sponsor.

Statistical Analysis Plan

Analytic Plan: The primary outcome measure will be standard deviation of lane position (SDLP), a measure of swerving. Each drive will yield two SDLP outcomes—one averaged across the entire drive, and one averaged across a short straight-away section within the drive. Both methods have been used in driving simulator research and thus, we will report both. The FDA recommends symmetry analysis to evaluate a drug's effect on the ability to operate a motor vehicle because analyses of mean changes may be insensitive to clinically meaningful impairment due to large inter-subject variability in PD effects[8]. Symmetry implies that, for any outcome, the probability of improvement (relative to the control) is the same as the probability of impairment on that outcome. The changes in SDLP (active day – control day) will be computed for each subject, and McNemar tests will be used to detect asymmetry in distributions. A review of the literature suggests that an increase in SDLP of 2.4cm relative to placebo is a sensible threshold for impairment as it reflects the impairment seen at a BAC of 0.05%[41, 28]. If SDLP changes after active dosing are random (i.e. the number of subjects with SDLP above +2.4cm and below -2.4cm are not different), then the active dose will be considered non-impairing. If an active dose causes more subjects to have SDLP changes above +2.4cm than below −2.4cm, that dose will be considered impairing. If a dose of alprazolam is found to be impairing by symmetry analysis, we will next conduct equivalency analyses to determine if that level of impairment is comparable to what is seen after zolpidem administration (and thus of potential regulatory significance). Secondary measures will be analyzed first as raw time-course data using three-factor models (dose x time x sex). For each outcome, peak scores (either minimum or maximum as appropriate) and time-to-peak will be calculated for each individual subject and dose condition (placebo condition excluded for time-to-peak analysis) and will be analyzed in a two-factor model (dose x sex). Models will be run in Proc Mixed and Tukey post hoc tests will examine significant main and interactions effects. Covariates may include body fat percentage, age, and sleep indices. As innovative statistical methods appear in the literature or updated guidance from regulatory or advisory agencies (e.g. FDA, ICADTS, etc.) are released, those methods will be incorporated into the analysis plan. Statistical analyses will be performed using SAS 9.3 and statistical significance will be set at $p<.05$. Based on our extensive experience conducting controlled clinical pharmacology studies, within-session

missing data are expected to be less than 3% for each outcome. Inspection of missing data and correlates of missingness will be examined upon study completion.

Sample Size and Power Analysis: Given the unique nature of this study and dearth of comparable studies on which to base sample size estimates, multiple power analyses were conducted to ensure adequate sample size and power. The first power calculation was premised on detecting a minimum treatment difference of 2.0cm SDLP (the primary outcome measure) with at least 90% power, assuming a within-subject SD of 2.1cm—this method has been successfully employed to detect next-day impairment of on-the-road driving after middle of the night hypnotic administration[28] with n=25. The second power analysis was based on the single study assessing potential next-day impairment of nighttime alprazolam administration[31], which found significant placebo vs. alprazolam effects on choice-reaction time (an outcome expected to be less sensitive than SDLP) with n=6. Together, these power analyses indicate than n=12 will be sufficient to detect significant main effects of drug compared to placebo; however, we are also interested in possible sex differences and thus propose to further increase power by completing 15 subjects per group for a total sample size of 30.