FOR OFFICE USE ONLY: IACUC Protocol # [redacted] Application Received: 12/14/18 Routed: 12/14/18 Training Complete:
Deadline to Request Full Committee Review: Covered Species? Yes Exempt Species? No Both? No IBC #

KANSAS STATE UNIVERSITY
University Research Compliance Office

Institutional Animal Care and Use Committee (IACUC)
Application for Approval Form

[redacted]

Version: Last Updated: 06/13/2016

ADMINISTRATIVE INFORMATION:

Responsible Individual/PI: [redacted]

Responsible Graduate Student (if applicable):

Title of Project/Course: The effects of fluconazole on methadone pharmacokinetics in dogs

Species/ Strain to be used: Dog

Type of Application: (check one box) ☐ New ☑ Addendum/Modification (complete modification block below)

Category: (check one box) ☐ Teaching ☐ Testing ☑ Research
☐ Other (if other, describe)

Funding Source: ☐ PHS/NIH ☐ Other Federal Agency ☐ State ☑ Other

Principal Investigator: [redacted] Degree/Title: [redacted]

Department: A&P Campus Phone: [redacted]

Campus Address: [redacted]

E-mail: [redacted] Alternate phone :

Co-Principal Investigators:

Name: [redacted] Dept: CS Degree/Title: DVM, PhD / Assoc. Professor

Name: [redacted] Dept: CS Degree/Title: DVM. MS / Clin. Assoc. Professor

MODIFICATION:

Is this a modification of an approved protocol? ☑ **Yes** ☐ **No** **If yes, please comply with the following:**

If you are requesting a modification or a change to an IACUC approved protocol, please provide a concise description of all of the changes that you are proposing in the following block. Additionally, please highlight or bold the proposed changes in the body of the protocol where appropriate, so that it is clearly discernible to the IACUC reviewers what and where the proposed changes are. This will greatly help the committee and facilitate the review.

Proposed modification 7
Add [redacted] to the protocol

Proposed modification 6
The proposed modification is to add four treatment groups as part of the crossover design (i.e. no additional dogs will be used). We also request the addition of [redacted] to the IACUC and please remove [redacted]

Group 10: ketamine IV, diazepam IV
Group 11: fluconazole PO, ketamine IV, diazepam IV
Group 12: ketamine IV, midazolam IV
Group 13: fluconazole PO, ketamine IV, midazolam IV

The purpose of this modification is to assess the effects of fluconazole on the plasma concentrations and effects of ketamine with diazepam and ketamine with midazolam as they are commonly used drug in the perioperative setting which is the expected use of the fluconazole/methadone combination product. Since the combination of fluconazole/methadone are likely to be used on the perioperative setting understanding the effects of fluconazole on other drugs is important.

Groups of 6 dogs each will be used (these are not additional dogs, but an additional crossover using the same dogs). The first proposed treatment addition (Group 10) will consist of ketamine (7 mg/kg) with diazepam (0.25 mg/kg) which will be administered IV as a bolus dose. Sedation, heart rate, respiratory rate, mucous membrane color and capillary refill time will be monitored every 5 minutes for the first 30 minutes, then every 10 minutes through the second hour, then every 15 minutes through the third hour then every 30 minutes through the fourth hour. The time from IV drug administration to standing will be recorded for each dog. Rectal temperature will be obtained every 15 minutes. Five blood samples per dog (3 mL per sample, 15 mL total volume) will be obtained at 5 and 10 minutes and at 2, 4 and 6 hours after drug administration by venipuncture for the determination of ketamine and diazepam plasma concentrations by liquid chromatography and mass spectrometry. Blood sample collection will occur after collecting physiologic data. Seven days later fluconazole will be administered (Group 11) at a dose of 5 mg/kg PO for 2 doses approximately 24 and 12 hours prior to administration of ketamine and diazepam as previously described. Sedation, physiologic measurements, time to standing and blood samples will be obtained in as previously stated.

The third proposed additional treatment (Group 12) will consist of ketamine (7 mg/kg) with midazolam (0.25 mg/kg) which will be administered IV as a bolus dose. Sedation, heart rate, respiratory rate, mucous membrane color and capillary refill time will be monitored every 5 minutes for the first 30 minutes, then every 10 minutes through the second hour, then every 15 minutes through the third hour then every 30 minutes through the fourth hour. The time from IV drug administration to standing will be recorded for each dog. Rectal temperature will be obtained every 15 minutes. Five blood samples per dog (3 mL per sample, 15 mL total volume) will be obtained at 5 and 10 minutes and at 2, 4 and 6 hours after drug administration by venipuncture for the determination of ketamine and midazolam plasma concentrations by liquid chromatography and mass spectrometry. Blood sample collection will occur after collecting physiologic data. Seven days later fluconazole will be administered (Group 13) at a dose of 5 mg/kg PO for 2 doses approximately 24 and 12 hours prior to administration of ketamine and diazepam as previously described. Sedation, physiologic measurements, time to standing and blood samples will be obtained in as previously stated.

Sedation Scale

0 - **No Sedation Present.**

1 - **Slight Sedation** - almost normal; able to stand easily, but appears somewhat fatigued, subdued or somnolent.

2 - **Moderate Sedation** - able to stand but prefers to be recumbent; sluggish; ataxic or uncoordinated.

3 - **Profound Sedation** - unable to rise, but can exhibit some awareness of environment; responds to stimuli through body movement; may be lateral or sternal recumbency.

4 - **Unresponsive** - in a state of anesthesia from which little or no response can be elicited; remains in lateral recumbency.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

*****Proposed modification 2*****
Submitted: 7/3/2017
Approved: 7/10/2017
The purpose of this modification is to change the dosing protocol dosing group 2. The initial protocol was: Group 2 - methadone with high dose fluconazole. Fluconazole will be administered at a targeted dose of 10 mg/kg PO q 12h for 3 doses. Methadone will be administered at a targeted dose of 1 mg/kg PO 2 hours after the last dose of fluconazole.

The proposed modified protocol is to change Group 2 and add Group 5.

Group 2 - single dose fluconazole. Fluconazole will be administered at a targeted dose of 5 mg/kg PO 12 hr prior to methadone dosing at 1 mg/kg PO at least 4 weeks after previous administration of fluconazole.

Group 5 - two doses of low dose fluconazole. Fluconazole will be administered at a targeted dose of 2.5 mg/kg PO 12 hr prior to methadone dosing at 1 mg/kg PO and concurrently with methadone dosing for 2 total doses of fluconazole at 2.5 mg/kg PO at least 4 weeks from previous administration of fluconazole.

The proposed modification is based on the sedation and decreased rectal temperatures that occurred in Groups 3 (methadone with intermediate dose fluconazole) and 4 (methadone with low dose fluconazole). The sedation and rectal temperature changes were indicative of

opioid mediated effects and as such there is no need to investigate high dose fluconazole with methadone. We would like to determine if a single dose prior to methadone is sufficient to produce similar effects on sedation and boy temperature and pharmacokinetic differences. Secondly we would like to determine if fluconazole administered at low dose (2.5 mg/kg PO) for a single dose prior to methadone administration followed by a dose administered concurrently with methadone will produce similar effects on sedation and boy temperature and pharmacokinetic differences. Both of these proposed groups will have a better translation into clinical practice (i.e. it is more reasonable to administer a single dose of fluconazole 12 hour prior to methadone and concurrently with methadone than starting 24 hours in advance of methadone treatment.

*****End of proposed modification 2*****

*****Proposed modification 1******
submitted: 4/28/17
approved:5/1/2017

The purpose of this modification is to give an either/or option to conduct the study in parallel (using 6 dogs/group, 1 treatment per group, 4 treatment groups for a total of 24 dogs included in the final study, assuming no drop-outs) as originally approved OR using an incomplete crossover design in which 12 dogs total are divided into 2 sets of 6 dogs and each set will receive a total of 2 treatments (i.e. 2 treatments per dog). If the crossover option is used at least a 1 week washout between the administration of methadone (alone) and methadone with fluconazole will be used. At least 4 weeks will be used as a washout period after treatments that include fluconazole to avoid carryover effects on drug metabolism. Previous studies with CYP inhibitors did not have carryover effects with a 4 week washout period (KuKanich et al 2011, KuKanich & KuKanich 2015), but specific data on fluconazole's effects on drug metabolism in dogs are not available.

*****End of Proposed modification 1******

I. NON-TECHNICAL SYNOPSIS (Please provide a brief narrative description of proposal. This should typically be less than 75 words and be easily understood by nonscientists, e.g. '*We propose to test the effectiveness of a new class of anti-inflammatory drugs against arthritis that develops in the hips of dogs affected by congenital hip dysplasia*'):

The purpose of this proposal is to determine if oral fluconazole administered with oral methadone to dogs will increase the amount of methadone absorbed into the blood stream and prolong the duration of effect. If successful, oral methadone may be used for pain control in dogs.

II. BACKGROUND (concise narrative review of the literature and basis for the study):

Methadone is an opioid that is effective for mild to severe acute pain and is routinely used in humans for analgesia. Methadone is approved for use in some countries for analgesia in dogs as an injection. Developing an oral dosing protocol for dogs will provide an effective means of controlling pain in outpatient or inpatient dogs without having to inject drugs. Methadone is well absorbed after oral administration, but the oral bioavailability is low due to rapid and extensive drug metabolism and inactivation prior to reaching systemic circulation. Administration of chloramphenicol significantly inhibits methadone metabolism resulting in good systemic drug exposure and prolonged clinical effects (KuKanich et al 2011; KuKanich & KuKanich 2015) . However chloramphenicol is an antimicrobial and administration may lead to selection and carriage of resistant bacteria. Additionally, chloramphenicol poses a risk of irreversible bone marrow suppression in humans, therefore it is not an ideal drug to enhance the oral bioavailability of methadone in dogs. A recent study demonstrated fluconazole inhibited drug metabolism in canine hepatocytes (in vitro) similar to chloramphenicol (Perez et al 2015). Fluconazole is an antifungal drug that does not select for bacterial resistance and does not have casual exposure risks to humans. Fluconazole is well tolerated in dogs (Mazepa et al 2011) with doses up to 10 mg/kg PO q 12 h routinely administered for treatment of fungal disease for up to and exceeding a year in duration at the Veterinary Health Center at Kansas State University. The investigators hypothesize co-administration of oral fluconazole and methadone will provide systemic methadone exposure to achieve prolonged clinical effects in dogs. The relevance of this study will provide a dosing regimen for a non-injectable opioid analgesic in dogs to treat moderate to severe pain with twice daily oral administration.

[redacted]

****Start Proposed modification 6****

The purpose of this modification is to assess the effects of fluconazole on the plasma concentrations and effects of ketamine with diazepam and ketamine with midazolam as they are commonly used drug in the perioperative setting which is the expected use of the fluconazole/methadone combination product. Since the combination of fluconazole/ methadone are likely to be used on the perioperative setting understanding the effects of fluconazole on other drugs is important.

****End proposed modification 6****

[redacted]

KuKanich B, KuKanich K. Chloramphenicol significantly affects the pharmacokinetics of oral methadone in Greyhound dogs. Vet Anaesth Analg. 2015 Nov;42(6):597-607.
Kukanich B, Kukanich KS, Rodriguez JR. The effects of concurrent administration of cytochrome P-450 inhibitors on the pharmacokinetics of oral methadone in healthy dogs. Vet Anaesth Analg. 2011 May;38(3):224-30.
[redacted]
Mazepa AS, Trepanier LA, Foy DS. Retrospective comparison of the efficacy of fluconazole or itraconazole for the treatment of systemic blastomycosis in dogs. J Vet Intern Med. 2011 May-Jun;25(3):440-5.
Perez T, Mealey K, Grubb T, Greene S, Court M.Tramadol metabolism to M1 and M2 in dog liver microsomes: interindividual variability, identification of responsible CYPS and drug-drug interactions. Proceedings of the 19th American Academy of Veterinary Pharmacology and Therapeutics Biennial Symposium. Fort Collins CO. 2015.

III. LITERATURE SEARCH FOR UNNECESSARY DUPLICATION

(If your proposed activity is part of the formal veterinary teaching curriculum and is not research or testing, you may not have to perform a literature search for unnecessary duplication. If it is teaching, please go to http://awic.nal.usda.gov/ for guidance on how to address Section III. A literature search for unnecessary duplication is required for all proposed research activities using animals.)

A. Date of literature search (should be within the last month): 10/23/2018

B. Search at least two appropriate databases and provide the years of coverage (i.e., PubMed (1950/current), CAB (1972/present)). A list of databases is available online at **http://www.lib.ksu.edu/db/subject/vetmed.html**:

1) Pub Med, 1950-present

2) CAB, 1950-present

3)

C. Keywords/Search Strategy:

****Proposed modification 6****
search terms fluconazole + dogs + ketamine; fluconazole + dogs + midazolam; and fluconazole + dogs + diazepam dogs
*****End Modification 6*****

Methadone + fluconazole + dog and/or methadone + fluconazole + dogs

D. Please provide a concise narrative of the results of the searches relative to unnecessary duplication. You do not need to provide a copy of the actual search with your proposal, but it should be maintained for your records or available to the IACUC if requested [redacted] e IACUC consultant. Please contact her if you need assistance. Phone [redacted]

****Modification 6****
No results in PubMed; CABI - 2 results, 1 was a formulary, the second was a case of blastomyces that underwent prolonged surgery and anesthesia that obscured any potential changes in the pharmacokinetics and effects of ketamine and diazepam
****End modification 6****

No results in either database

IV. OBJECTIVE\HYPOTHESIS (briefly state the objective of the study - and, if applicable, the hypothesis to be accepted or rejected):

****Modification 6****
A secondary objective is to assess the effects of fluconazole on two commonly administered drug combinations: ketamine/diazepam and ketamine/midazolam. The hypothesis is that no to minimal changes will occur in the degree of sedation in the treatment groups with fluconazole compared to the groups without fluconazole. However the duration to standing will be increased in the groups administered fluconazole.
****End modification 6****

The objective of this study is to assess the pharmacokinetics of oral methadone and methadone with escalating doses of fluconazole. The hypothesis is fluconazole will cause a dose-dependent increase the drug exposure as measured by the area under the curve and maximum plasma concentration.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures (succinctly outline formal scientific plan for study):

This will consist of 28 Beagle dogs divided into 4 groups of 7 dogs using a parallel study design. Six dogs will be included in each group with 1 dog per group in the event one of the original 6 does not complete the study. Group 1 and Group 2 will be completed first and if clinical opioid effects are observed or plasma drug concentrations confirm methadone plasma concentrations than Groups 3 and 4 will be completed.

Alternatively 12 Beagle dogs will be used in an incomplete crossover design. Dogs will be randomly divided by gender into 2 sets of six dogs. Each set will receive 2 treatments. Set 1 will receive methadone with no fluconazole followed by methadone with high dose fluconazole with at least a 1 week washout period between treatments. Set 2 will receive methadone with low dose fluconazole and methadone with intermediate dose fluconazole with at least 4 weeks between treatments.

Group 1 - methadone with no fluconazole. Methadone will be administered at a targeted dose of 1 mg/kg PO.
Group 2 - methadone with high dose fluconazole.
Fluconazole will be administered at a targeted dose of 5 mg/kg PO 12 hr prior to methadone dosing at 1 mg/kg PO.
Group 3 - methadone with low dose fluconazole. Fluconazole will be administered at a targeted dose of 2.5 mg/kg PO q 12h for 3 doses. Methadone will be administered at a targeted dose of 1 mg/kg PO 2 hours after the last dose of fluconazole.
Group 4 - methadone with intermediate dose fluconazole. Fluconazole will be administered at a targeted dose of 5 mg/kg PO q 12h for 3 doses. Methadone will be administered at a targeted dose of 1 mg/kg PO 2 hours after the last dose of fluconazole.
Group 5 - two doses of low dose fluconazole. Fluconazole will be administered at a targeted dose of 2.5 mg/kg PO 12 hr prior to methadone dosing at 1 mg/kg PO and concurrently with methadone dosing for 2 total doses of fluconazole at 2.5 mg/kg PO.
Group 6 - Methadone with fluconazole. Fluconazole will be administered at a targeted dose of 5 mg/kg PO with methadone at 1 mg/kg PO in a liquid suspension for 2 doses separated by 12 hours

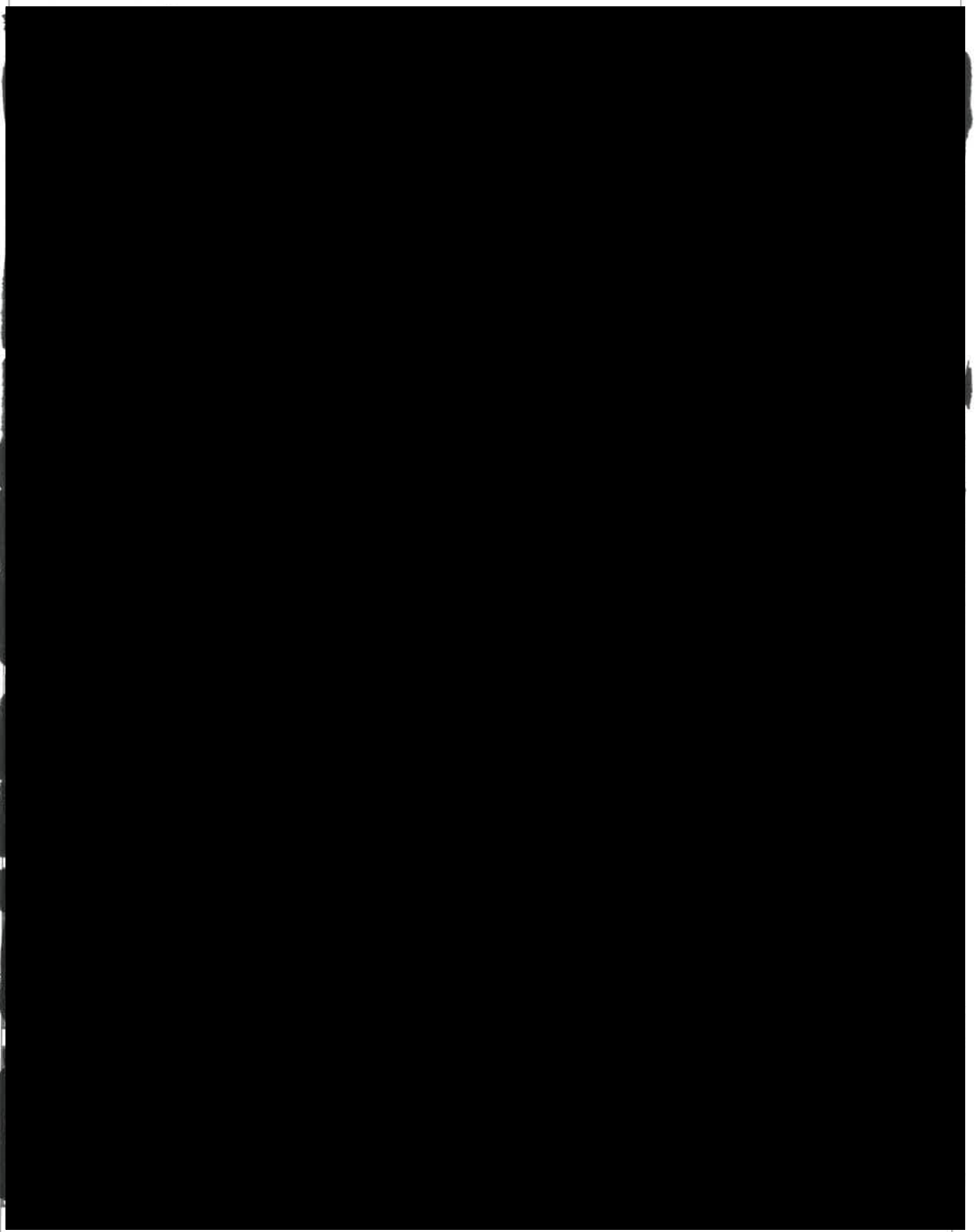
[redacted]

At least 12 hours prior to methadone administration, jugular catheters will be placed with sedation available if needed (acepromazine 0.01-0.02 mg/kg IV/IM/SC; butorphanol 0.2 mg/kg IV or 0.4 mg/kg IM/SC). Jugular catheters are routinely placed in well tempered dogs without sedation (Riel, 2010), but since these dogs are not as well accustomed to routine handling and restraint, sedation may be needed. The investigator (B KuKanich) has extensive experience placing jugular catheters in awake dogs, however with the number of dogs enrolled in this study it is possible some dogs will not have the temperament for jugular catheter placement. The area over the jugular vein will be clipped of hair, and scrubbed with alternating chlorhexidine followed isopropyl alcohol three times. A small amount of 2% lidocaine (1 mL) with sodium bicarbonate (0.1 mL) will be infused at the insertion site of the catheter to numb the area. The catheter will then be placed and a protective wrap consisting of sterile pads with antibiotic ointment, cling gauze, and vet wrap will be applied.

For treatment groups 1-5
Blood samples (10 samples, 4 mL each) will be obtained prior to methadone administration (time 0) and at 20 and 40 minutes and at 1, 2, 4, 6, 8, 12 and 24 hours after methadone administration. No more than 40 mL of whole blood will be collected from each dog per treatment. The dogs are expected to have at least a minimum body weight of 4 kg which will have at most a total blood volume collected of 10 mL per kg of body weight (1%) which is within a volume not expected to cause physiologic changes. Blood samples will be collected from an aseptic jugular catheter. In the case of premature catheter removal or malfunction, venipuncture (20-22 g x 1" needle attached to a 5-6 mL syringe) will be used to collect blood. For collection of blood from the jugular catheter, 1 mL of blood will be withdrawn into a syringe containing 1 mL 0.9% saline with 5 IU heparin/mL. Blood, 4 mL will then be collected for drug analysis. The 1 mL blood volume with 0.9% saline with heparin will be administered through the jugular catheter, then the catheter will be flushed with 5 mL 0.9% saline. Each dog will receive 50 U of heparin far below the heparin dose (70 U/kg) administered to dogs for systemic anticoagulant effects.

Pharmacodynamic measurements will be collected prior to obtaining blood samples and at times 0, 1, 2, 4, 6, 8, 12, and 24 hours. Sedation will be assessed using a categorical scale: none - no apparent effect; mild - drowsy, but still active; moderate - drowsy, glazed eyes, but still able to walk without assistance; heavy - very drowsy, unable to walk or requires assistance to walk. Heart rate will be obtained by thoracic auscultation for 30 seconds and respiratory rate will be collected over 30 seconds. Rectal temperature will then be obtained, followed by the blood sample as previously described. Heart rate, respiratory rate

and rectal temperature will be compared statistically between the treatment groups at each time point using a t-test (Sigma Stat 12.5, Systat Software Inc. CA, USA) if normally distributed and of uniform variance. Sedation will be compared statically between the treatment groups using the Mann-Whitney rank sum test (Sigma Stat 12.5, Systat Software Inc. CA, USA



Groups of 6 dogs each will be used (these are not additional dogs, but an additional crossover using the same dogs). The first proposed treatment addition (Group 10) will consist of ketamine (7 mg/kg) with diazepam (0.25 mg/kg) which will be administered IV as a bolus dose. Sedation, heart rate, respiratory rate, mucous membrane color and capillary refill time will be monitored every 5 minutes for the first 30 minutes, then every 10 minutes through the second hour, then every 15 minutes through the third hour then every 30 minutes through the fourth hour. The time from IV drug administration to standing will be recorded for each dog. Rectal temperature will be obtained every 15 minutes. Five blood samples per dog (3 mL per sample, 15 mL total volume) will be obtained at 5 and 10 minutes and at 2, 4 and 6 hours after drug administration by venipuncture for the determination of ketamine and diazepam plasma concentrations by liquid chromatography and mass spectrometry. Blood sample collection will occur after collecting physiologic data. Seven days later fluconazole will be administered (Group 11) at a dose of 5 mg/kg PO for 2 doses approximately 24 and 12 hours prior to administration of ketamine and diazepam as previously described. Sedation, physiologic measurements, time to standing and blood samples will be obtained as previously stated.

The third proposed additional treatment (Group 12) will consist of ketamine (7 mg/kg) with midazolam (0.25 mg/kg) which will be administered IV as a bolus dose. Sedation, heart rate, respiratory rate, mucous membrane color and capillary refill time will be monitored every 5 minutes for the first 30 minutes, then every 10 minutes through the second hour, then every 15 minutes through the third hour then every 30 minutes through the fourth hour. The time from IV drug administration to standing will be recorded for each dog. Rectal temperature will be obtained every 15 minutes. Five blood samples per dog (3 mL per sample, 15 mL total volume) will be obtained at 5 and 10 minutes and at 2, 4 and 6 hours after drug administration by venipuncture for the determination of ketamine and midazolam plasma concentrations by liquid chromatography and mass spectrometry. Blood sample collection will occur after collecting physiologic data. Seven days later fluconazole will be administered (Group 13) at a dose of 5 mg/kg PO for 2 doses approximately 24 and 12 hours prior to administration of ketamine and diazepam as previously described. Sedation, physiologic measurements, time to standing and blood samples will be obtained as previously stated.

****End of modification 6****

Reference:
Riel, DL. Jugular catheterization and central venous pressure. in Textbook of Veterinary Internal Medicine 7th edn. Ettinger SJ & Feldman EC Eds. Saunders Elsevier, St Louis, MO. 2010 pp. 317-319.

B. Non-animal Alternatives Considered (were non-animal alternatives considered - why are they not used?):

In vitro studies identifying fluconazole as inhibiting the same canine drug metabolizing as chloramphenicol have already been completed at a different university. Therefore the next step is to assess of the same drug interaction occurs *in vivo* that was identified *in vitro*.

C. Animal Model and Species/Strain Justification (Explain why animals are needed for your study. Give your rationale and justification for selecting this animal model or species):

Dogs are the target population.

D. Animals Requested -used in research testing or teaching (list genus and species/strain of animal model proposed):

Genus and Species:	dog - canis faniliaris
Total number (by species) requested: (this should correspond to the sum of the animals listed in Section VI.A. below. The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the TOTAL number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.)	Dogs-28 total
Source of animals (by species):	Dogs: 28 KSU owned [redacted]

E. Justification of Animal Numbers / Data: Analysis: Research, testing, and teaching activities should be designed to provide a statistically significant result with a minimum number of animals. The specific method by which the number of animals was determined must be clearly stated. Statistical techniques and/or power analysis are appropriate in most cases to maximize the usefulness of the data generated from each animal. However, the IACUC acknowledges that the basis for an appropriate justification of animal numbers depends largely on the nature of the study itself. Prior experience and expertise with the model in question may be taken into account as well, but must be carefully documented in the protocol. The cost of the animal should not be considered as the primary justification for the use of a particular species or model. Consultation with a biostatistician or use of statistical software during the design phase of the experiment may be useful. This website may be helpful in performing a power analysis: **http://statpages.org**

Five basic types of studies are listed below, along with brief general guidelines for the justification of animal numbers appropriate for each type of study. These guidelines are intended to provide direction - any given study may not fall neatly into one of these five categories. **Select the appropriate box(es)** below and supply a narrative explanation that will clearly explain your rationale and justification for the number of animals proposed for your activity:

☐ 1. **Teaching Protocols:** (Animal numbers are determined by a specified student-to-animal ratio, which must be explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without sacrificing the quality of the hands-on teaching experience for students).

☐ 2. **Tissue Harvest Required for *In-vitro* Work and / or Antibody Production:** (Animal numbers are determined by the amount of tissue required and the number of individual animals needed to provide the appropriate amount of tissue, antibodies, etc. A detailed explanation of how the required number of animals was determined must be included in the justification narrative).

☐ 3. **Exploratory Study Requiring No Statistical Analysis - Qualitative**: (use of live animals to demonstrate success or failure of a desired goal, such as the production of transgenic mice): Animal numbers are justified based on the probability of success of the experimental procedure; a detailed explanation of how that probability was determined must be included in the narrative).

☑ 4. **Pilot Studies**: (Animal numbers are determined by the investigator's experience and personal judgment, and are typically small. Data collected in pilot studies are generally used to determine statistically relevant sample size calculations for future experiments).

Typically 6 animals/group are used in pharmacokinetic studies based on the investigators experience and current literature. However since these are unfamiliar dogs and may not be trained to this study design it is anticipated that not all dogs in each group will complete the study and as such a 7th animal per group is requested (4 groups at 7 animals per group = 28 animals). Use of a larger number of animals, n=28, and use parallel versus crossover study design is due to decreasing multiple use and subsequent stress in an individual animal (i.e. at most each dog will only go through 1 sedation and blood sample collection). Additionally, the duration of drug metabolism inhibition by fluconazole is unknown and as such a crossover study may result in a carry over effect of drug metabolism inhibition.

Alternatively 12 dogs will be used in an incomplete crossover study design with dogs divided into 2 sets of 6. In order to minimize the potential of carryover effects of fluconazole on drug metabolism, set 1 will receive methadone without fluconazole followed by methadone with high dose fluconazole (no chance of fluconazole metabolism inhibition carryover) with at least a 1 week washout period. Set 2 will receive methadone with low dose fluconazole and methadone with intermediate dose fluconazole with at least 4 weeks between treatments to minimize potential carryover of fluconazole metabolism inhibition. Previous studies have indicated 4 weeks was a sufficient period of time to minimize risk of carryover effects on drug metabolism by other inhibitors (ketoconazole, chloramphenicol, cimetidine, fluoxetine, trimethoprim and medetomidine) in dogs (KuKanich et al 2011, KuKanich & KuKanich, 2015), but specific data on the length of drug metabolism inhibition by fluconazole are not available.

☐ 5. **Studies Requiring Inferential Statistical Analysis**: (If possible, animal numbers are determined by statistical power analysis; the justification statement must include the specific test, i.e., ANOVA, student t-test, chi square, etc., used to determine sample size. Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant).

☐ a. **Statistical Test:**

☐ b. **Literature Reference:**

1. Reference- (provide specific reference(s) for numbers justification)

2. Narrative Justification- (provide a succinct justification / rationale for using the reference(s) to determine the numbers proposed in the activity)

☐ 6. **Other**: (This applies if your activity does not fit into one of the other categories. If you check this option, you must provide a detailed and defendable description of the rationale for the number of animals proposed for your activity).

VI. HUMANE CONSIDERATIONS:

A. Pain Category (for your proposal, please estimate the number of animals in each applicable pain category below to the best of your knowledge - it may be appropriate to list animals in more than one pain category, i.e. controls in Cat. C, infected animals in Cat. D or E. If more than one species is requested, provide pain category estimates on all species requested. We are required to report this animal use and pain category information annually to the USDA).

USDA Pain and/or Distress Category

Please estimate the number of animals in your proposed activity that would fall into one or more of the following three pain and/or distress categories. It is common to have animals listed in more than one category - for example, an uninfected control versus a challenge group. The cumulative total number for the three Pain Categories should equal the total number of animals requested in Section V.D.

SPECIES #1 (common name):	Dog	
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	28
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

(If you are using more than one species in this activity, also complete the following section)

SPECIES #2 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	
SPECIES #3 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

If more species are used, please list them on an attached sheet.

* List animals in USDA Pain Category B that are being bred, conditioned or held for use.

* List animals in USDA Pain Category C that will undergo no activity that will produce pain and/or distress, or procedures similar to those that might routinely be performed on humans by a physician without provision of anesthesia or analgesia, i.e. injections, phlebotomy, ear tagging, etc. If you only listed animals in category B or C, you may skip Sections VI.B-F below and resume with Section VI.G.

** List animals in USDA Pain Category D that will undergo procedures where pain-alleviating methods are used, such as anesthesia, analgesia. Surgical patients would fall into this category, even if the procedure were terminal. If you placed animals in Category D or E, you must carefully complete Section VI. B-D below

*** List animals in USDA Pain Category E that will experience unalleviated pain and/or distress. This should be considered only when the use of a pain alleviating strategy would seriously compromise the validity of the study, and/or no other option is available or possible. If you place animals in Category D or E, you must carefully complete Section VI.B-D below.

The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the **TOTAL** number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.

B. **Alternatives to Painful Procedures** (If you have animals listed in Pain Category D or E above, you must provide the following information. The Animal Welfare Act requires that you provide a narrative description of methods used and sources searched to ensure that you have verified that alternatives are not available to prevent unnecessary pain and distress. The Animal Welfare Information Center (AWIC) has a site that gives tips for performing this search http://www.nal.usda.gov/awic/alternatives/tips.htm [redacted] the IACUC consultant. Please contact her if you need assistance. [redacted]

1. **Date of literature search** (should be within the last month):

2. **Search at least two appropriate databases and provide the years of coverage** (i.e., PubMed (1950/current), CAB (1972/present). A list of databases is available online at http://www.lib.ksu.edu/db/subject/vetmed.html:

   1)

   2)

   3)

3. **Keywords/Search Strategy:**

4. **Concise Narrative:**

C. **Painful Procedure Justification** (How do you plan to minimize unnecessary pain and/or distress? You must provide strong justification for having animals in Category D or E above):

D. **Attending Veterinarian Consultation:** ☑ **Yes** ☐ **No**

Name: [redacted] Date Contacted: 02/20/2017

If you have animals listed in Pain Category D or E in paragraph VI.A. above, the AWA requires that you formally consult with the IACUC attending veterinarian (AV) or his designee on all aspects of pain and / or distress management. This must be done prior to submission of the pr[redacted] consultation, please contact [redacted]
*Important note: the AV consult is not the IACUC review of your proposal. Please understand that the IACUC committee is autonomous and members will likely ask different questions they deem appropriate during the actual committee review.

E. **Prolonged Restraint:** ☐ **Yes** ☑ **No** (Describe and justify any plans for prolonged restraint >15 min. Reference IACUC Guideline #2)

F. **Pain or Distress Alleviation** - Will you be administering drugs or compounds for sedation, anesthesia or analgesia as a premedication or for anesthetic induction or maintenance? ☐ **Yes** ☑ **No** **(If "YES", all animals receiving the drug or compound will need to be placed in USDA Pain Category D.)**

1. List all drugs or compounds being used for sedation, anesthetic or analgesia during the course of your procedure. Included drug/compound name, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency

2. How will you monitor the animal to ensure the animal is properly anesthetized?

G. **Surgery** ☐ **Yes** ☑ **No**

(Reference IACUC guidelines #4, #10)

1. **Procedure** (Describe surgical procedures planned)

2. **Location** (Where is the surgical procedure to be performed?)

3. **Surgeon/Qualifications** (Who will perform procedures? List their training and qualifications.)

4. **Multiple Survival Surgery Procedures** ☐ **Yes** ☐ **No** (If yes, please provide justification)
(Reference IACUC guideline #7)

5. **Non-Survival Surgery Procedures** ☐ **Yes** ☐ **No**

H. **Animal Monitoring** - For protocol purposes, a procedure is defined as an action performed on an animal for research or teaching purposes that has the potential to cause pain or distress to that animal. In order to evaluate pain and/or distress, the KSU IACUC requires an approved plan of how pain or distress will be minimized and documentation of how observations of animals will be recorded.

All procedures performed upon an animal should be listed on an **Animal Monitoring Plan (AMP)** form which is submitted with your IACUC protocol. The AMP form along with the **Animal Observation Record (AOR)** detail how you will observe your animals and what actions you will take in order to minimize pain or distress associated with your research project. Examples of when theses forms would be required include animals that undergo a surgical procedure, animals that undergo anesthesia, animals experimentally infected with an infectious disease, or animals inoculated with potential tumor forming cells. Exceptions to the use of the AMP and AOR would be simple procedures with minimal physiological effect upon the animal, examples of which include vaccination, blood collection, or injection of experimental compounds.

Please complete and submit the AMP with the Protocol application. A link to these forms along with further directions can be found at the KSU IACUC home page. Since the IACUC may follow up on compliance with this requirement, you should maintain these records with your study records after the end of the research project.

If an AMP is included in my approved IACUC document, I understand that it is my responsibility as the PI to assure that the AMP activities will be used as described in the approved protocol. I also understand that should oversight bodies request them, it is my responsibility to be able to document the activities called for in the AMP.

1. Does this protocol require the use of the AMP and AOR? ☑ **Yes** ☐ **No**
   (Checking "YES" will make the AMP form appear on the next page)
2. Is an AMP completed? ☑ **Yes** ☐ **No**
3. Indicate where the AMP will be kept (i.e. animal room posted on wall, lab or barn office).

   animal room posted and office

Animal Monitoring Plan

Protocol #: [redacted] PI: [redacted] PI Contact #: [redacted]

Animal/Group ID: Species: Dogs Animal Location: [redacted]

Procedure: Jugular catheter placement Date of Procedure: TBD

I. Post-Procedure Care (if applicable)

A. List all drugs/medications to be given following the procedure (include name, dose, route, and frequency)

Drug/Medications	Dose	Route	Frequency

B. List all other care to be provided following the procedure and note frequency.

Post-Procedure Care	Frequency
Monitor catheter site for pain	q 12h
Body temperature	q 12h
Check bandage	q 12h
Monitor sedation	q 1 h until able to stand on own

II. Observations

A. Observation Frequency: q 1h initially, then q 12h. For vF device monitor q 12 hours

B. When will the animal be returned to its cage/pen:

After catheter placement

C. List the parameters to be monitored, criteria to monitor for and directions for recording, and the appropriate action to be taken if necessary.

Parameter	Monitoring Criteria	Intervention
Monitor catheter site for pain	Vocalization, avoidance	Remove bandage, check catheter
Body temperature	>103F	Check catheter, remove if swollen, red, hot, painful
Check bandage	Check bandage placement	Replace bandage if needed
Monitor sedation	Check ability to stand. Check mucous membrane color. Check heart rate (HR).	In unable to stand, continue to monitor. If mucous membranes are not pink or capillary refill time exceeds 3 seconds notify veterinarian on call If HR <40 or >180 notify veterinarian on call
[redacted]	[redacted]	[redacted]

III. Contact Information:

	Name	Telephone Number
PI	[redacted]	[redacted]
Co-Investigator	[redacted]	[redacted]
Co-Investigator	[redacted]	[redacted]
Veterinarian	[redacted]	[redacted]

In the event that the investigators or the responsible veterinarian cannot be reached or if you have concerns about an animal's care, please contact the [redacted]

I. **Animal Manipulations:**

1. **List all other drugs and compounds** that you will be administering other than those listed above in Pain or Distress Alleviation (Section F), on the Animal Monitoring Plan (Section H) or in Euthanasia (Section J.8). Include drug, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency
Fluconazole (10 or 40 mg/mL suspension or 50 or 100 mg tablets)	2.5, 5 or 10 mg/kg	PO	q 12h
Methadone (5 or 10 mg tablets)	1 mg/kg	PO	once
Butorphanol (10 mg/mL)	0.2-0.4 mg/kg	IV/IM/SC	once PRN
Acepromazine (10 mg/mL)	0.01-0.02 mg/kg	IV/IM/SC	once PRN
Lidocaine (2%) with sodium bicarbonate (8.4%)	20 mg total dose lidocaine 0.1 mEq sodium bicarbonate	SC	Once
[redacted]	[redacted]	[redacted]	[redacted]
Fluconazole/methadone (see above for formulations/stock drugs)	5 mg/kg / 1 mg/kg	PO	q 12h
[redacted]	[redacted]	[redacted]	[redacted]
Ketamine Diazepam	7 mg/kg 0.25 mg/kg	IV IV	once once
Ketamine Diazepam Fluconazole	7 mg/kg 0.25 mg/kg 5mg/kg	IV IV PO	once once q12h (twice)
Ketamine Midazolam	7 mg/kg 0.25 mg/kg	IV IV	once once
Ketamine Midazolam Fluconazole	7 mg/kg 0.25 mg/kg 5mg/kg	IV IV PO	once once q12h (twice)

2. **List any rooms where procedures with animals are done (excluding housing and surgery).** Locations for procedures such as behavior testing, treadmill training, blood draws, injections, gavage, etc. should be listed in this chart. If procedures are performed within CMG facilities, the "CMG assigned".

Building/Room Number	Procedure
[redacted]	Catheter Placement, pharmacokinetic study

3. **Biosamples:** ☑ **Yes** ☐ **No** (list type & amount, i.e., phlebotomy, minor biopsies, ascitic fluids, etc.)

Jugular catheters: 3-12" ; 16-20 gauge. Clip hair. Surgical site prep, alternating chlorhexidine and isopropyl alcohol scrubs x 3 each. Cover catheter insertion with sterile pad with antibiotic ointment and then wrap with cling gauze and finally vet wrap. Blood samples: 38 - 46 mL total volume per dog per dog (10-15 kg dogs). If catheter fails or is prematurely removed, phlebotomy will be performed using a 3/4 - 1" ; 20-22 gauge needle attached to a 3 cc syringe.

4. **Tissue Sharing:** ☐ **Yes** ☑ **No** (detail any tissue sharing you plan with other investigators)

N/A

5. **Other Procedures:** (list any other procedures you might perform on animals in this project)

N/A

6. **Adjuvants:** ☐ Yes ☑ No (explain any adjuvant use. Reference IACUC guideline #12)

7. **Chemical Grade Drugs:** ☐ Yes ☑ No (If you plan to use a chemical grade please list and provide a scientific explanation for its use; **Reference IACUC guideline # 19)**

J. Veterinary Care:

1. **Animal Housing:** (Provide specific information on where the animals will be housed for your activity.)
PLEASE INCLUDE ROOM NUMBER IF KNOWN

[redacted]

2. **Social/Paired Housing:** (Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility. "The Guide" 8th Edition):

☐ Yes ☑ No My animals will be housed in stable pairs or compatible groups?

If no, please provide an adequate justification for an exception to this guidance.

The dogs will be single housed for the study to minimize premature catheter removal during the study.

3. **Special Husbandry Considerations:** (Animals will be housed in designated animal rooms/areas, unless approved by the IACUC. Detail special husbandry requirements, i.e. special diets, micro-isolators, etc.):

N/A

4. **Animal Surveillance:** (Who observes the animals daily for health problems?)

The investigators will observe the animals at least twice daily. All investigators are veterinarians with experience in monitoring dogs.

5. **Veterinary Clinical Care:** (Who will you contact if there is a health problem requiring veterinary care?)

CMG veterinarian

6. **Wire Bottom Rodent Caging:** If you are using rodents, do you propose to house them in wire-bottom cages?

☐ Yes ☐ No (If yes, you must explain the rationale for the use of wire bottom cages scientifically. See IACUC Guideline #14)

☑ N/A

7. **Study Endpoint** (Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The IACUC must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, expected and possible adverse effects research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints since they minimize pain and distress. **The use of death of the animal as an endpoint is strongly discouraged and must be justified to the IACUC - Reference IACUC guideline # 13**. Please describe the endpoint of your study):

This is a survival study. Severe adverse effects are not expected due to the large safety margin of the drugs being evaluated.

8. **Euthanasia:** (Reference the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition, link available on the KSU IACUC or the AVMA website, https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx)

Will animals be euthanized as a part of your protocol? ☐ Yes ☑ No

i. **Method** (include drug, dosage, and route)

ii. **Name of person(s) responsible for performing the euthanasia.**

9. **Animal Disposition** (what is your plan for the animals after the study is over?)

☐ **Euthanasia** ☐ **Adoption** ☐ **Long-term holding**

☑ **Transfer to another investigator with approved or pending protocol.**

Name: [redacted]

☐ **Other**

VII. **Investigator & Technician Qualifications/Training** (The Animal Welfare Act and the PHS Policy requires that personnel are appropriately trained in animal care and use matters, and that the professional training is documented. The PI is responsible for ensuring that all study personnel have completed appropriate professional training. **Prior to final approval of an animal care and use protocol, the IACUC requires completion of the required activity specific online training for all personnel listed as participating in the animal care and use activity.** The URCO will have access to documentation of completion of the online training through CITI. All other training documentation is the responsibility of the PI. List all persons involved in your activity below - excluding [redacted] for information or guidance on animal care and use training)

Name	Training and experience with animals
[redacted]	DVM, 7 years of small animal practice experience as a veterinarian, 12 years of laboratory animal research experience with dogs, cats, horses, cattle and exotic animal species
[redacted]	DVM, 15 years of small animal practice experience as a veterinarian (intern, resident and boarded internal medicine specialist) with dogs, cats and a variety of other species
[redacted]	DVM, > 20 years of clinical practice experience as a veterinarian (including internship, residency and is currently a boarded anesthesiologist) with dogs, cats, horses, cattle and a variety of other species
[redacted]	DVM, surgeon, 7 years veterinary experience including internship and residency training in surgery with dogs, cats and some other animals species
[redacted]	Licensed Veterinary Technician - 12 years of experience as veterinary technician in the shelter medicine program (College of Veterinary Medicine's Shelter Medicine Mobile Surgery Unit)

The IACUC is required to review and approve changes in personnel for research or teaching involving animals. **Consequently, you must inform the IACUC (via protocol modification) of any changes in animal care research personnel that may occur in your activity. Additionally, you must ensure that new personnel involved in your activity are qualified, have completed the mandatory animal care and use training, and are enrolled in the occupational health and safety program.

☑ Yes ☐ No **Will personnel be trained in humane handling of this species?**

☑ Yes ☐ No **Are all personnel enrolled in the KSU Animal Worker Occupational Health and Safety Program?**

(If no, forms can be downloaded from http://www.k-state.edu/research/comply/iacuc/ohsp or you may contact the [redacted]

☐ Yes ☑ No **Will you need animals for protocol-related training purposes, i.e., experimental or surgical technique development or refinement, etc.?** If yes, please specify the technique or procedure to be performed during training (you may reference detailed description in another section of the proposal if appropriate):

Number of animals required to accomplish the proposed training (be sure to include the number of animals requested for training purposes in the total number of animals listed in Section V.D., and Section VI.A.):

Please indicate how training is/will be accomplished:

- ☑ Yes ☐ No **Training and/or orientation with P.I., CMG or LACS personnel**
- ☑ Yes ☐ No **Instruction by supervising animal caretaker**
- ☑ Yes ☐ No **Viewing of instructional videos**
- ☐ Yes ☑ No **Other (please specify)**

☑ Yes ☐ No **Individual Technical Procedure Training Form.**

if you marked no, explain below how you are going to document training or technical competence for personnel to perform the procedure(s) proposed.

If you are proposing to use a technical, manipulative, or invasive procedure on animals as part of your activity, it is a requirement that you document the competence of your staff to perform the proposed procedure. Documentation of training is necessary for all personnel for specific animal use procedures such as handling, stomach tubing, euthanasia, injections, biopsy, phlebotomy, restraint, etc. This formal training documentation should be maintained in the laboratory or close by and be readily available for IACUC, USDA, AAALAC, OLAW and research compliance review as appropriate. It is the PI's responsibility to ensure that adequate training is performed, and documented. If you need assistance with training for technical procedures, contact the [redacted]

VIII. Hazardous Material Use: (explain if are you using hazardous materials in your study)

1. ****Biological, Infectious or Parasitic agents** ☑ No ☐ Yes (list)
2. ****Recombinant or synthetic nucleic acid molecules** ☑ No ☐ Yes (list)
3. **Hazardous chemicals** ☑ No ☐ Yes (list)
4. **Radioisotopes** ☑ No ☐ Yes (list)
5. **Other** ☑ No ☐ Yes (list)
6. **Select Agents:** Are you using or planning to use agents listed in the Federal Select Agent Program. (http://www.selectagents.gov/SelectAgentsandToxinsList.html)?

☑ No ☐ Yes (list)

The Federal Select Agent Program (www.selectagents.gov), a joint program of the Centers for Disease Control and Prevention (CDC), and the USDA Animal and Plant Health Inspection Service (APHIS), oversees the activities of possession, use and transfer of biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products. The program currently requires registration of facilities including government agencies, universities, research institutions, and commercial entities that possess, use or transfer biological agents and toxins.

If you plan to use or are using any of the viruses, bacteria, fungi, rickettsial agents, or toxins on the select agent list, please contact the [redacted]

(If "yes" you must have a Registration Document from the Institutional Biosafety Committee)**

IBC Registration Document # _____ **Approval Date** _____

IX. Extramural Funding: (It is critical that animal care and use procedures detailed in the IACUC protocol are consistent with external funding proposals documents. Discrepancies between the two documents in animal care and use procedures could jeopardize individual and/or institutional funding and compliance. If you make changes, or they are required by the IACUC, it is your responsibility to ensure that grant or funding agencies are informed.)

☐ Yes ☐ No All animal care and use procedures described in this proposal are consistent with those described in external funding applications/documents. If no is checked, please contact the [redacted]

☑ N/A

X. **Clinical Research:** (Does this activity involved client owned animals with naturally occurring, or pre-existing conditions?)

☐ **Yes** ☑ **No**

XI. **USDA Regulated Activities:** (Is your activity regulated by provisions of the Animal Welfare Act?) Contact the URCO or the attending veterinarian if you need clarification.

Regulated animals would include: - Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or warm-blooded animal used for biomedical research, teaching, testing, experimentation, or exhibition purposes. Exemptions to this definition are listed below.

Exempt or non-USDA regulated animals would include: (1) lab rats and mice (*Mus / Rattus*) bred for use in research, (2) birds, (3) horses not used for (biomedical) research purposes, and (4) other farm animals such as, livestock or poultry, used or intended for use as food or fiber, or improving animal nutrition, breeding, management, production efficiency, or for improving food or fiber quality.

☑ **Yes** - My activity involves species COVERED by the definition of animal in the Animal Welfare Act.
☐ **No** - My activity involves animals that are **EXEMPT** from coverage by the USDA
☐ **Both** - My activity involves both covered and exempt species.
☐ **Also** - My activity involves NIH Regulated Activities (use of any **vertebrate species**).

XII. **Wildlife or Field Investigation:**

☐ **Yes** ☑ **No** Does your activity involve the use or observation of nondomesticated vertebrate species under field conditions?

If "YES," please answer the following:

☐ **Yes** ☐ **No** Does your wildlife field activity require any international, federal, state or local permits?

☐ **Yes** ☐ **No** Are you using any relevant professional society guidelines that are available for your wildlife field activity ?

Online Required Training

TRAINING REQUIREMENTS HAVE RECENTLY CHANGED

The IACUC requires mandatory training prior to protocol approval. Training is now offered through the Collaborative Institutional Training Initiative (CITI) Program. Instructions to register and access training are found on the URCO website: http://www.k-state.edu/research/comply/

Use the check boxes below to select the training courses that apply to this protocol. If you have any questions about training, contact [redacted]

Mandatory Training

Required for all Principal Investigators, research staff and students

☒ Responsible Conduct of Research ☒ Working with the IACUC

Required (Provost-mandated) for all full time K-State employees

☒ Export Compliance

Species-specific training (check all that apply to this protocol)

☐ Swine	☐ Cattle	☐ Rat	☐ Mouse	☐ Guinea Pig	☐ Hamster	☐ Ferret
☑ Dog	☐ Cat	☐ Horse	☐ Gerbil	☐ Sheep or Goat	☐ Rabbit	☐ Zebrafish
☐ Fish (except zebrafish)		☐ Amphibians	☐ Wildlife (except fish)	☐ Farm Animals or Agricultural Animals		

Required procedure-specific training (check all that apply to this protocol)

☐ Survival Surgery

☐ Rat or Mouse, Category D or E procedures

☐ Antibody Production

All new personnel or personnel with expired training are required to register for CITI and take the new training requirements. If you previously completed online IACUC modules, your training status will remain current until it expires. URCO will verify training from the previous system as well as the new system prior to approval of any protocol.

POST APPROVAL MONITORING: The URCO has a Post-Approval Monitoring (PAM) program to help assure that animal care and use activities are performed in accordance with provisions or procedures approved by the IACUC. Accordingly, the URCO staff will arrange PAM visits as appropriate to assess compliance with approved activities.

INVESTIGATOR ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS FOR TEACHING AND RESEARCH

(Print this page separately because it requires a signature by the PI.)

P.I. Name: [redacted]

Title of Project: The effects of fluconazole on methadone pharmacokinetics in dogs

XIII. ASSURANCES: As the Principal Investigator on this protocol, I provide assurances for the following:

A. **Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, and in accordance with applicable laws, regulations, and guidelines. Any deviation or modification from the procedures detailed herein, must receive prior approval from the Institutional Animal Care and Use Committee (IACUC).**

B. **Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.**

C. **Statistical Assurance: I assure that there has been an adequate evaluation of the experimental design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.**

D. **Oversight: All experiments, surgeries, or manipulations involving live animals will be performed under my supervision or that of another qualified individual. In procedures involving USDA Pain Category D or USDA Pain Category E, I have consulted with the attending veterinarian on minimizing pain and/or distress.**

E. **Biohazard\Safety: I assure that in planning this proposal, I have made the proper consideration regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant DNA issues, etc. Additionally, personnel on my study with contact with animals are enrolled in the Animal Worker Occupational Health and Safety Program.**

F. **Training: I assure that personnel performing animal procedures\manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures\manipulations. Inexperienced personnel will be properly trained and/or supervised. Additionally, I understand that I must maintain documentation of appropriate animal care and use training for personnel involved in my study.**

G. **Adverse Event Notification: In compliance with provisions of both the "Ag" and "ILAR Guide," I assure that I will notify the IACUC Attending Veterinarian (Dr. Marlow) if there is a significant unanticipated adverse event during the execution of my activity. This would include unexpectedly high levels of mortality or development of a new disease condition that affects the health and / or welfare of the animals, etc.**

H. **Extramural Funding: If funded by an extramural source, I assure that this application accurately reflects all procedures involving laboratory animal subjects as described in the proposal to the funding agency. (standards are the same, regardless of funding sources).**

I. **Study Duration: I understand that proposals are approved for 3 years. I also understand that as subsequent annual reviews are conducted, it is my responsibility to provide timely and accurate annual review information when requested, to include notification of the IACUC and the University Research Compliance Office (URCO) when my study is completed.**

You may sign this form using a digital signature. DO NOT sign the form until it has been completed.
You cannot edit the form entries once the form has been digitally signed. If you are making revisions to a previously signed form, right-click the digital signature and select Clear to remove the signature (this can only be done by the person who originally digitally signed the form). Forms that have not been signed will not be accepted.

P.I. Signature: [redacted]