Department of Veterans Affairs

Memorandum

Date: [redacted]

From: [redacted]

Subj: Pending Approval of Triennial Review Submission (PCC #: 2017-060556)

To: Chair, GLA Institutional Animal Care and Use Committee (IACUC) (151)

Re: ***Breeding Protocol for Normal and Narcoleptic Dogs***
VA Project #: **0016**
Protocol #: **9902-002**
Sponsor: **0000 - None**

Thank you for the review. Below are my responses to each of the comments:

ITEM 1: Section C, Number 1. There are a lot of grammatical errors in this section. Please contact [redacted] for assistance with correcting this section and other similar problems elsewhere in the ACORP.

This section has been revised following the advice of [redacted]

ITEM 2: Section C, Number 2b. Section C, Number 1 indicates there will be only 1-2 pairs of breeders, and have no plans to breed now due to funding issues. However, Section C, Number 2b indicates more animals and has breeding. Please reconcile.

Section C, Number 2b has been revised to match that of Number 1.

ITEM 3: Section D. Explain why rat, mouse, or other lower animal models cannot be used instead of dogs.

This paragraph has been revised to reflect the fact that narcoleptic dogs are unique in terms of the etiology of the disease (receptor mutation vs deletion of cells), the similarity of symptoms to human narcoleptics (readily inducible cataplexy), and possiblity of studying CSF hypocretin.

ITEM 4: Section H. This section indicates dogs will be obtained from Team Associates in Pennsylvania. Congress was just informed that all the dogs on this protocol are all bred here. Please clarify and confirm with the veterinarian that this is an approved vendor. Also, this vendor does not show up on a Google search.

This line has been deleted. We have no immediate plane to breed normal dogs. If we find the need in the future, we will obtain them from a USDA approved vendor.

ITEM 5: Section J. Check the "This protocol includes category D procedures" box.

The box has been checked.

ITEM 6: Section N. Check the box for "Inside of VMU".

The box has been checked.

ITEM 7: Section O. Check the "No" box.

The "No" box has been checked.

ITEM 8: Section R. Remove CSF from the table.

CSF colleciton has been removed from the table.

ITEM 9: Section T. Include how often are the dogs are weighed.

It's indicated now that dogs are weighed weekly.

ITEM 10: Section U. Correct the spelling of "cessation" in the table.

"Cessation" has been corrected.

ITEM 11: Section U, Number 3. Change the wording to "...euthanizing dogs with this method".

The sentences have been revised as suggested.

ITEM 12: Section W. Correct the spelling of C-section.

"C-section" has been corrected.

ITEM 13: Section W, Number 1. Do an ALTBIB search with the search terms dog, intra-uterine insemination

A search on ALTBIB with the search terms dog, intra-uterine insemination was done and came up with no results.

ITEM 14: Section W, Number 1. Do an ALTBIB search with the search terms dog, C-section

A search on ALTBIB with the search terms dog and C-section was done and no alternative procedures was found.

[redacted]
PCC 2017-060556
[redacted]

ITEM 15: Section W, Number 1. The search for "narcolepsy, mouse, rat" will only bring up papers with both mice and rats. [redacted] with assistance on this search.

[redacted]**has been consulted. A search with "narcolepsy" and "mouse or rat" and "hypocretin" and "induced cataplexy" was done and no induced cataplexy equivalent to narcoleptic dogs was found in rodent models.**

ITEM 16: Appendix 1. The Appendix 1 indicates [redacted] will perform the survival surgery. Isn't the clinic doing the survival surgery? Please clarify and/or rectify.

The task code "B" denoting survival surgery has been removed.

ITEM 17: Appendix 5, Number 3. Add the surgery numbers to the table.

Surgery numbers have been added to the table.

ITEM 18: Appendix 5, Number 4. Add "TBD by private clinic" in the "Room Number" column.

"TBD by private clinic" has been added in the"Room Number" column.

[redacted]

Department of Veterans Affairs

Memorandum

Date: [redacted]

From: Chair, GLA Institutional Animal Care and Use Committee (IACUC) (151)

Subj: Pending Approval of Triennial Review Submission (PCC #: 2017-060556)

To: [redacted]hD (151A7)

Re: ***Breeding Protocol for Normal and Narcoleptic Dogs***
VA Project #: **0016**
Protocol #: **9902-002**
Sponsor: **0000 - None**

1. The above referenced Triennial Review submission was reviewed by the IACUC using the Designated Review process on July 6, 2017 and ***requires the following contingencies be satisfied before approval is granted.***

ITEM 1: Section C, Number 1. There are a lot of grammatical errors in this section. Please contact D[redacted] for assistance with correcting this section and other similar problems elsewhere in the ACORP.

ITEM 2: Section C, Number 2b. Section C, Number 1 indicates there will be only 1-2 pairs of breeders, and have no plans to breed now due to funding issues. However, Section C, Number 2b indicates more animals and has breeding. Please reconcile.

ITEM 3: Section D. Explain why rat, mouse, or other lower animal models cannot be used instead of dogs.

ITEM 4: Section H. This section indicates dogs will be obtained from [redacted]
Please clarify and confirm with the veterinarian that this is an approved vendor. [redacted]

ITEM 5: Section J. Check the "This protocol includes category D procedures" box.

ITEM 6: Section N. Check the box for [redacted]

ITEM 7: Section O. Check the "No" box.

ITEM 8: Section R. Remove CSF from the table.

ITEM 9: Section T. Include how often are the dogs are weighed.

ITEM 10: Section U. Correct the spelling of "cessation" in the table.

ITEM 11: Section U, Number 3. Change the wording to "…euthanizing dogs with this method".

ITEM 12: Section W. Correct the spelling of C-section.

ITEM 13: Section W, Number 1. Do an ALTBIB search with the search terms dog, intra-uterine insemination

ITEM 14: Section W, Number 1. Do an ALTBIB search with the search terms dog, C-section

ITEM 15: Section W, Number 1. The search for "narcolepsy, mouse, rat" will only bring up papers with both mice and rats. [redacted]

ITEM 16: Appendix 1. The Appendix 1 indicate [redacted] will perform the survival surgery. Isn't the clinic doing the survival surgery? Please clarify and/or rectify.

ITEM 17: Appendix 5, Number 3. Add the surgery numbers to the table.

ITEM 18: Appendix 5, Number 4. Add "TBD by private clinic" in the "Room Number" column.

2. **Your resubmission must include a) the revised documents, b) a copy of this memorandum, and c) a memo detailing the changes you have made in response to each contingency on an item-by-item basis. Your response must be submitted electronically via email and as a signed, paper copy to the Investigator Services Center (ISC).** The following options are available to respond to each contingency:
   a. If you agree to meet a contingency, make the appropriate changes in the submission and specify the exact changes in your response (do not simply say "done").
   b. If you disagree with the committee's interpretation of the regulations or understanding of the procedures you wish to perform, state your disagreement and its basis.
   c. If you believe that there are valid scientific reasons that a requested change would compromise your research, present the reasons or request to appear at a committee meeting to discuss the issues.
3. **Please note that approval of this protocol must be completed before July 15, 2017 or it will expire.**
4. If you have questions about this submission, please contact the IACUC Coordinator [redacted]

[redacted]

ANIMAL COMPONENT OF RESEARCH PROTOCOL (ACORP) [redacted]

Main Body
VERSION 4 v2 6-17-2015

See Instructions for Completion of the Animal Component of Research Protocol (ACORP Instructions), for help in completing specific items.

A. **ACORP Status.**

1. Full Name of Principal Investigator(s [redacted]
2. VA Station Name (City) and 3-Digit Station Number ► **VA Greater Los Angeles 691**
3. Protocol Title ► **Breeding Protocol for Normal and Narcoleptic Dogs**
4. Animal Species covered by this ACORP ► **Dog**
5. Funding Source(s). Check each source that applies:

   ►(x) Department of Veterans Affairs.
   ►( ) US Public Health Service (e.g. NIH).
   ►( ) Private or Charitable Foundation -- Identify the Foundation:
   ►( ) University Intramural Funds – Identify the University and Funding Component:
   ►( ) Private Company – Identify the Company:
   ►( ) Other – Identify Other Source(s):

6. Related Documentation for IACUC reference.

   a. If this protocol applies to a project that has already been submitted to the R&D Committee for review: ► **NO** (x) **-- GO TO Item #7**
   Else, identify the project:
   (1) Title of project ►
   (2) If approved by the R&D Committee, give the date of approval ►

NOTE TO REVIEWERS: Part 6b differs from version 3 ACORP. This is basically a progress report. Reviewers should consider if this project has been inactive and flag that for discussion by committee.

   b. Triennial review. If this protocol is being submitted for triennial *de novo* review, complete the following:
   (1) Identify the studies described in the previously approved ACORP that have already been completed
   ► **N/A**

   (2) Indicate the numbers of animals of each breed/strain/genotype that have already been used, and adjust the numbers shown in Item I accordingly

   ► **Three pups were born in the last three years.**

   (3) Describe any study results that have prompted changes to the protocol, and briefly summarize those changes, to guide the reviewers to the details documented in other Items below.

► **N/A**

c. List any other relevant previously approved animal use protocols (copy the lines below as needed for each protocol listed).
(NOTE TO REVIEWERS: This is section would only be filled out under specific circumstances. This section should normally be left blank)
(1) Title of other protocol ►
(2) IACUC approval number of other protocol ►
Give the name of the VA station or other institution that approved it, if it was not approved by the IACUC that will review this ACORP ►

7. Indicate the type(s) of animal use covered by this protocol (check all that apply):
►( ) Research
►( ) Teaching or Training
►( ) Testing
►(x) Breeding and colony management only; not for any specific research project
►( ) Holding protocol (as specified by local requirements; not required by VA, PHS, or USDA)
►( ) Other. Please specify►

Proposal Overview

B. **Description of Relevance and Harm/Benefit Analysis.** Using non-technical (lay) language that a senior high school student would understand, briefly describe how this research project is intended to improve the health of veterans, the general population and/or other animals, or otherwise to serve the good of society, and explain how these benefits outweigh the pain or distress that may be caused in the animals that are to be used for this protocol.

(NOTE TO REVIEWERS: Please check that benefits to the veteran population is specifically addressed in this section)

► Narcolepsy is a sleep disorder primarily caused, in humans, by the loss of a particular type of cells in the hypothalamus of the brain. These cells normally produce a chemical called hypocretin (Hcrt; also called orexin). It is not clear how these cells die, but it may be a result of interactions between the immune system and the brain. The narcoleptic dog is the best characterized animal model of narcolepsy. These dogs have a genetic mutation in a receptor for hypocretin rather than a loss of the cells as in human narcoleptics. However, it has all the major symptoms of human narcolepsy, including excessive daytime sleepiness, loss of muscle tone (cataplexy) and symptom onset after birth. Manipulations of the hypocretin system, the immune system, and the brain need to be validated in the animal model before they can be used in humans to treat or prevent this disease. Studying the narcoleptic dogs has the advantage of being able to apply experimental manipulations that are impossible in humans. Cracking the link between hypocretin system and narcolepsy in the dog should shed light on both the normal function of the hypocretin system and the pathophysiology of this system in human narcolepsy. The purpose of this project is to maintain a small colony of narcoleptic and normal dogs. The breeding program allows us to continue research programs that have direct relevance for studies of the cause and treatment of human narcolepsy and are likely to have broader impact on a wide variety of sleep disorders. Sleep disorder is becoming more prevalent in the aging general population. Sleep-related health problems are even more prevalent in the veteran population (Mustafa et al., Sleep Breath, 2005;

Ocasio-Tascón et al., Sleep Breath, 2006) which is further complicated by post-traumatic syndrome and a higher tendency for obesity, high blood pressure, and chronic alcohol consumption. Our research on narcoleptic dogs should further our knowledge on the cause of narcolepsy and sleep disorder in general. Our research should also help the development of treatment for sleep disorder and thus benefit our aging veteran population.

C. **Experimental Design.**

1. **Lay Summary**. Using non-technical (lay) language that a senior high school student would understand, summarize the conceptual design of the experiment in no more than one or two paragraphs.

► **We have downsized our dog colony to maintain 1-2 pairs of breeders. We don't have plan to breed more now due to funding constraints. We may start to breed again when the financial situation improves. When it does, we will monitor all females for signs of heat twice a week starting one month before their normal seasonal estrus. We will try natural breeding first by housing males and females together. However, both male and female narcoleptic dogs often have cataplexy during intercourse which greatly reduces the chances of successful insemination. In the past we have had our greatest success breeding the narcoleptic dogs with artificial insemination, and we will do artificial insemination if natural breeding fails. We will use natural breeding for the normal dogs. To find the best time for insemination, blood will be drawn up every other day to measure progesterone levels in female dogs once they are in heat.**

Once the time of ovulation is determined based on levels of progesterone and luteinizing hormone artificial insemination will be performed on the female. Both frozen and fresh semen will be used to maintain genetic diversity. Two to three inseminations will be made with 1-2 days apart.

Ultrasound examination for pregnancy will be performed on the female about one month following insemination.

The condition and temperature of the female will be monitored every 4 hours starting one week before the due date. Continuous monitoring will begin when the temperature drops below 100 °F until the delivery. The puppies and mother will be kept together until weaning at 5-6 weeks of age. Animals may also have blood drawn for periodic health exams.

2. **Complete description of the proposed use of animals.** (NOTE TO REVIEWERS: This section is similar but not exact to ACORP Version 3. Please note the new layout).

Use the following outline to detail the proposed use of animals.

a. **Summarize** the design of the experiment in terms of the specific groups of animals to be studied.

► **We are breeding narcoleptic and control dogs to study of the cause of narcolepsy and brain mechanisms responsible for narcolepsy. We will use frozen semen in addition to fresh semen from the male breeders to maintain genetic diversity. Signs of heat, e.g., vaginal discharge, will be monitored twice a week starting one month before their normal seasonal estrus. Once discharge is detected, blood will be drawn every other day for analysis of progesterone level as an indicator for ovulation. The level of progesterone will progressively increase as the bitch**

approaches ovulation. A progesterone level of 4-8 ng/ml is an indication of approaching ovulation and thus best time for insemination in the next few days.

We will try natural breeding first. If the males are not capable of mating then artificial insemination will be done. Intravaginal insemination procedures will be performed by members of the Siegel laboratory, using a standard pipette. Transcervical and intra-uterine inseminations will be done at an IACUC approved private clinic. Intra-uterine procedure will only be done if all other methods fail. The bitch and 1-2 male dogs will be transported to the clinic and back the same day using the VMU van.

Pregnancy will be assessed by palpation and ultrasound around 28 to 30 days after insemination. Ultrasound examination will also be done at the clinic. Physical and behavioral changes of the bitch, e.g., swelling of the nipples and vulva plus "nest building" behavior, at later days will further confirm pregnancy. The bitch will be brought into a room and temperature measured 2 times daily at least one week before the due date (average of 63 days from conception). Twelve to 24 hours before she is due to deliver, the dam's rectal temperature may drop from 101 to 98 degrees. Clear discharge from the vulva may occur. The bitch will be put in a whelping room at lest 48 hr before the due date. The condition and temperature of the bitch will be checked at least 3 times/day. The puppies and mother will be kept together until weaning at 6 weeks of age.

If complications occur during delivery, the dam will be taken to the clinic. C-section may be performed if deemed necessary.

b. **Justify the group sizes and the total numbers of animals requested.** A power analysis is strongly encouraged; see ACORP instructions.

► **We have minimized our dog colony to 3 dogs. There is no immediate plan to breed more due to budget constraints. We may start to breed again in the future depending on the financial situation and the experimental needs.**

c. **Describe each procedure** to be performed on any animal on this protocol. (Use Appendix 9 to document any of these procedures that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.) Describe each procedure in a few words or just one sentence, and then write "see appendix X for details". e.g. stereotaxic surgery – see appendix 5 for details, Morris water maze – see appendix 6 for details.

(NOTE TO REVIEWERS: This section is a significant change in the previous ACORP. Note that *departures from the Guide* are to be listed here and in appendix 9. This section will likely require significantly more attention from the VMO to identify departures from the GUIDE.

► **Semen collection, transcervical insemination, intra-uterine insemination and C-section are performed in a private clinic which we have been using for 28 years and is inspected by the IACUC twice a year.**

Semen collection
Fresh semen will be collected from stud dogs periodically (to be frozen for future use) and on the day of insemination at the private clinic. This is done by massaging the bulbus glandis until

erection and eventually ejaculation. A female in heat is usually brought in to facilitate the process. It normally takes less than 5 min for the stud to ejaculate. The 2nd fraction of the ejaculate is rich in spermatozoa. A drop of semen is first evaluated under the microscope for viability. Semen is then spun for 2 min and the top prostate fluid discarded. Frozen semen will be obtained from the clinic. These semen samples have been collected from our dogs periodically in the past and stored in liquid nitrogen tank.

Intravaginal insemination
Either fresh semen or frozen semen will be used. Semen is gently infused through a pipette to the deepest part of the vagina at the junction of the cervix. A second and occasionally third insemination is performed, usually 1 to 2 days apart.

Transcervical insemination
Transcervical procedure is accomplished with the aid of a rigid fiberoptic endoscope and a urinary or angiographic catheter, on the standing bitch, and without sedation. Semen is gently infused through a pipette into the opening of cervix. A second and occasionally third insemination is performed, usually 1 to 2 days apart.

Intra-uterine insemination
With the dam under general anesthesia and in dorsal recumbency, the ventral abdomen is clipped, and after routine surgical preparation a 4 - 6 cm incision is made midway between the pubis and the umbilicus, through the linea alba. The uterus is elevated through the incision, and the needle of the syringe containing the semen is inserted into the lumen of the uterine body at a 45° angle with the bevel of the needle up. The semen is slowly injected into the uterus. Buprenorphine (0.01 mg/kg, IM) will be given twice daily for 3 days post op.

C-section
C-section is considered when complication occurs or is expected to occur during natural delivery of puppies. Standard C-section procedures will be followed with the dam under general anesthesia. A local anesthetic is administered around the incision after the abdominal wall is closed. The dam will be allowed to nurse the puppy immediately after recovery to increase the bond which tends to be weaker with C-section as compared to natural delivery. Buprenorphine (0.01 mg/kg, IM) will be given twice daily for 3 days post op.

D. **Species.** Justify the choice of species for this protocol.
► The narcoleptic dog is the best understood animal model for narcolepsy. In the dog, narcolepsy has all the major features of human narcolepsy, including excessive daytime sleepiness, cataplexy, and symptom onset after birth. Narcoleptic dogs are different from mouse and rat models in that cataplexy can be induced with play or palatable food, similar to the situation in human narcoleptic patients that cataplexy can be triggered by laughter or strong emotions. In rodent models spontaneous cataplexy can only be enhanced but not induced. In addition, in the dog narcolepsy is not caused by the absence of hypocretin cells but rather is due to a mutation in one of the hypocretin receptors. This provides us a unique opportunity to study the potential different mechanisms in terms of how hypocretin deficiency causes narcolepsy. Because hypocretin cells are intact it is also possible to study hypocretin release in the CSF. We have been successfully breeding these animals for the last 25 years. We have developed techniques for recording brain and other physiological activities from these animals.

Personnel

E. **Current qualifications and training.** (For personnel who require further training, plans for additional training will be requested in Item F.)

1. PI
Name ▶
Animal research experience ▶ [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

Specific procedure(s) that the PI will perform personally	Experience with each procedure in the species described in this ACORP
Intravaginal insemination	[redacted]

2. Other research personnel (copy the lines below for each individual)
(NOTE TO REVIEWERS: Each person on the ACORP should be discussed here. Qualifications should likely be described in general terms—[euthanasia, surgery, feeding, etc, rather than specifics, but the IACUC committee should decide level of experience. Likely, more complex procedures/techniques will require more specific descriptions.)
Name ▶ **N/A**
Animal research experience ▶ [redacted]
Animal research experience ▶ [redacted]

Qualifications to perform specific procedures

Specific procedure(s) that this individual will perform	Experience with each procedure in the species described in this ACORP
Assisting intravaginal insemination	[redacted]

Gavaging the milk for the puppy	[redacted]

3. VMU animal care and veterinary support staff personnel (copy the lines below for each individual)
   Name► **To be determined by VMO** (NOTE TO REVIEWERS: This should always be "TBD BY VMO) and will be pre-populated)

Qualifications to perform specific support procedures in the animals on this protocol

Specific support procedure(s) assigned to this individual	Qualifications for performing each support procedure in the species described in this ACORP (e.g., AALAS certification, experience, or completion of special training)
TBD BY VMO	TBD BY VMO

4. For each of the research personnel listed in items 1 and 2 above, enter the most recent completion date for each course. [redacted]

Name of Individual	Working with the VA IACUC	ORD web-based species specific course (Identify the species)	Any other training required locally (Identify the training)
[redacted]		[redacted]	

F. **Training to be provided.** List here each procedure in Item E for which anyone is shown as "to be trained", and describe the training. For each procedure, describe the type of training to be provided, and give the name(s), qualifications, and training experience of the person(s) who will provide it. If no further training is required for anyone listed in Item E, check box "N/A"
► ( ) **N/A**
► Additional training:

G. **Occupational Health and Safety.**

1. Complete one line in the table below for each of the personnel identified in Item E:

Name	Enrollment in Occupational Health and Safety Program		Declined optional services	Current on Interactions with OHSP? (yes/no)
	VA program	Equivalent Alternate Program – identify the program		
[redacted]	(x)	( )	( )	
	(x)	( )	( )	

2. Are there any non-routine OHSP measures that would potentially benefit, or are otherwise required for, personnel participating in or supporting this protocol?

► ( ) Yes. Describe them ►

► (x) No.

Animals Requested

(NOTE TO REVIEWERS: This section is basically the same as previous version of the ACORP)

H. **Animals to be Used.** Complete the following table, listing the animals on separate lines according to any specific features that are required for the study (see ACORP Instructions, for guidance, including specific terminology recommended for the "Health Status" column):

Description (include the species and any other special features not shown elsewhere in this table)	Gender	Age/Size on Receipt	Source (e.g., Name of Vendor, Collaborator, or PI of local breeding colony)	Health Status
Dog/narcoleptic Doberman	**M/F**	**0 – 10 yrs/ up to 50 kg**	[redacted]	**Conventional**

I. **Numbers of animals requested.** See ACORP Instructions, for descriptions of the categories and how to itemize the groups of animals.

USDA Category B

Procedures►						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category B TOTAL**

USDA Category C

Procedures► breeding/blood collection						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category C TOTAL**
Dog/Normal female Doberman	**1**	**1**	**1**			**1***
Dog/normal male Doberman	**1**	**1**	**1**			**1***
Dog/narcoleptic female Doberman	**1**	**1**	**1**			**1***
Dog/narcoleptic male Doberman	**1**	**1**	**1**			**1***

*The same sogs are used year after year

USDA Category D

Procedures► breeding/Intra-uterine insemination/C-section (only if needed –otherwisr the animal will be in Category C)						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category D TOTAL**
Dog/narcoleptic female Doberman	**1**	**1**	**1**			**1**

USDA Category E

Procedures►						
Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Category E TOTAL**

TOTALS over all Categories

Species / strain	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**GRAND TOTAL**
Dog/normal Doberman	**2**	**2**	**2**			**2***
Dog/narcoleptic Doberman	**3**	**3**	**3**			**3***

*** Note: The same dogs will be used year after year.**

J. **Management of USDA Category D procedures**. Indicate which statement below applies, and provide the information requested.

► ( ) This protocol does NOT include any Category D procedures.

► (x) This protocol INCLUDES Category D procedures. List each Category D procedure and provide the information requested. (For surgical procedures described in Appendix 5, only identify the procedure(s) and enter "See Appendix 5 for details.)

Procedure	Monitoring (indicate the method(s) to be used, and the frequency and duration of monitoring through post-procedure recovery)	Person(s) responsible for the monitoring	Method(s) by which pain or distress will be alleviated during or after the procedure (include the dose, route, and duration of effect of any agents to be administered)

Intra-uterine insemination	**Continuous monitoring of blood pressure, heart rate and oxygen saturation with pulse oximeter; check for body temperate with rectal probe, and gum color every 15 min until ambulatory**	**Private clinic staff**	**Anesthetics:** **Propofol (4.0 mg/kg, i.v.; induction dose)** **Isoflurane (1.5-5%, inhalation; maintenance dose, until completion of surgery)** **Analgesics:** **2% Lidocaine around the incision line** **Buprenorphine (0.01 mg/kg, IM) twice daily for 3 days post op.**
C-section	**Continuous monitoring of blood pressure, heart rate and oxygen saturation with pulse oximeter; check for body temperate with rectal probe, and gum color every 15 min until ambulatory**	**Private clinic staff**	**Anesthetics:** **Propofol (4.0 mg/kg, i.v.; induction dose)** **Isoflurane (1.5-5%, inhalation; maintenance dose, until completion of surgery)** **Analgesics:** **2% Lidocaine around the incision line** **Buprenorphine (0.01 mg/kg, IM) twice daily for 3 days post op.**

K. **Justification of Category E procedures.** Indicate which statement below applies, and provide the information requested.

► (x) This protocol does NOT include any Category E procedures

► ( ) This protocol INCLUDES Category E procedures. Identify each Category E procedure included in this ACORP and justify scientifically why the pain or distress cannot be relieved.

►

Veterinary Care and Husbandry

L. **Veterinary Support.**

1. The laboratory animal veterinarian who is responsible for ensuring that the animals on this protocol receive appropriate veterinary medical care.

(NOTE TO REVIEWERS: This section will be prepopulated)
[redacted]

2. Veterinary consultation during the planning of this protocol.

   Name of the laboratory animal veterinarian consulted ► [redacted]
   Date of the veterinary consultation (meeting date, or date of written comments provided by the veterinarian to the PI) ► [redacted]

M. **Husbandry.** As a reference <u>for the animal husbandry staff</u>, summarize here the husbandry requirements of the animals on this protocol. (Use Appendix 6 to justify the use of any special husbandry and to detail its effects on the animals. Use Appendix 9 to document any aspects of the husbandry that involve "departures" from the standards in the *Guide*. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.) (NOTE TO REVIEWERS: This is a potentially confusing section that is a significant departure from previous ACORPs. Please be aware of non-standard housing and *<u>departures from the GUIDE</u>*. The VMO should provide extra attention to this section. Areas that may be important in this section are housing of breeding pairs, weaning of babies, and housing of animals singly for experimental reasons.)

1. Caging needs. Complete the table below to describe the housing that will have to be accommodated by the housing sites for this protocol:

a. Species	b. Type of housing*		c. Number of individuals per housing unit**	d. Is this housing consistent with the *Guide* and USDA regulations? (yes/no***)		e. Estimated maximum number of housing units needed at any one time
Dog	(x)	**Departures from the Guide**	1-3	(x)	**No**	**6**

*See ACORP Instructions, for guidance on describing the type of housing needed. If animals are to be housed according to a local Standard Operating Procedure (SOP), enter "standard (see SOP)" here, and enter the SOP into the table in Item Y. If the local standard housing is not described in a SOP, enter "standard, see below" in the table and describe the standard housing here:

► (x) **Standard (See SOP)—Enter SOP in the table in Item Y.**
► ( ) **Standard (not covered by a SOP)**
► **Describe:**

** The *Guide* states that social animals should generally be housed in stable pairs or groups. Provide a justification if any animals will be housed singly (if species is not considered "social", then so note)

► (x) **N/A: Animals will be housed in stable pairs or groups .**
► (x) Animals will be housed singly:

►Provide justification: **Animals may be incompatible or of the wrong sexes to prevent or facilitate breeding.**

***Use Appendix 9 to document "departures" from the standards in the *Guide*.

2. Enrichment. Complete the table below to indicate whether "standard" exercise and environmental enrichment will be provided to the animals on this protocol, or whether any special supplements or restrictions will be required (See ACORP Instructions, for more information on enrichment requirements. Use Appendix 9 to document any enrichments requirements that represent "departures" from the standards in the *Guide*.):

(NOTE TO REVIEWERS: This section will be prepopulated, as the VMO will determine appropriate enrichment)

a. Species	b. Description of Enrichment*		c. Frequency	
Dog	(x)	**TBD By VMO**	(x)	**TBD By VMO**
		Non-standard enrichment (describe and justify below)		**Other**

*If enrichment will be provided according to a local SOP, enter "standard (see SOP)" and enter the SOP into the table in Item Y. If the local standard enrichment is not described in a SOP, enter "standard, see below", and describe the standard species-specific enrichment here. (NOTE TO REVIEWERS: In those cases where experimental design or other factors preclude use of standard enrichment, the PI may need to fill out the "non-standard box").

► (x) **Standard (TBD by VMO)**
► ( ) **Non-standard**
Description on non-standard enrichment and justification:

3. Customized routine husbandry. Check all of the statements below that apply to the animals on this protocol, and provide instructions to the animal husbandry staff with regard to any customized routine husbandry needed.

► ( ) This ACORP INCLUDES genetically modified animals.

List each group of genetically modified animals, and describe for each any expected characteristic clinical signs or abnormal behavior related to the genotype and any customized routine husbandry required to address these. For genetic modifications that will be newly generated on or for this protocol, describe any special attention needed during routine husbandry to monitor for unexpected clinical signs or abnormal behavior that may require customized routine husbandry.

►

► (x) This ACORP does NOT include use of any animals that will require customized routine husbandry. If checked, go to item N.

► ( ) Devices that extend chronically through the skin WILL be implanted into some or all animals on this protocol. Describe any customized routine husbandry to be provided by animal husbandry staff to minimize the chances of chronic infection where the device(s) penetrate the skin.

► (x) **N/A**

► ( ) **Describe:**

► ( ) Some or all of the animals on this protocol WILL require other customized routine husbandry by the animal husbandry staff, beyond what has been described above. Describe the special husbandry needed.

► (x) **N/A**
► ( ) **Describe:**

N. **Housing Sites.** Document in the tables below each location where animals on this protocol may be housed. (NOTE TO REVIEWERS: All VMU space should be noted as TBD BY VMO.)

► (x) Housing on VA property. Identify each location on VA property where animals on this protocol will be housed, and indicate whether or not each location is inside the VMU. If it will be in the VMU, just indicate West LA VMU or Sepulveda VMU.

Building	Room number	Inside of VMU?	
		Yes	No
[redacted]	TBD by VMO	x	

► ( ) Housing in non-VA facilities. Identify each location not on VA property where animals on this protocol will be housed, and provide the information requested in the table. (NOTE TO REVIEWERS: This space is for [redacted].

Name of Non-VA Facility	Is this facility accredited by AAALAC?		Building	Room Number
	Yes -- enter status*	No**		
		( )**		
		( )**		
		( )**		

*See ACORP Instructions, for a list of AAALAC accreditation status options.

**For any facility listed above that is not accredited by AAALAC, attach documentation that a waiver has been granted by the CRADO.

Special Features

O. **Antibody Production.** Will any of animals on this protocol be used for the production of antibodies?

► ( ) Some or all of the animals on this protocol WILL be used in the production and harvesting of antibodies. Check "Appendix 2" in Item Y, below, and complete and attach Appendix 2, "Antibody Production".
► (x) NO animals on this protocol will be used in the production and harvesting of antibodies.

P. **Biosafety.** Will any substances (other than those used in routine husbandry or veterinary care) be administered to the animals on this protocol?

►( ) This protocol INVOLVES administration of substances to the animals other than those used in routine husbandry and veterinary care. Check "Appendix 3" in Item Y, below, and complete and attach Appendix 3, "Biosafety".
►(x) This protocol does NOT involve administration of any substances to the animals other than those used in routine husbandry and veterinary care.

Q. **Locations of procedures.** Complete the table below, listing the location(s), inside or outside of the animal facility, for each of the procedures to be performed on animals on this protocol.

Procedure	Surgical?		Bldg/Room Number	Requires transport between the VMU and the laboratory, or transport between laboratories?	
	Yes	No		No	If Yes – describe method of discreet transport
Blood collection	( )	(x)	[redacted]	(x)	
Semen collection	( )	(x)		( )	[redacted]
Intravaginal insemination	( )	(x)		(x)	
Transcervical insemination	( )	(x)		( )	
Intra-uterine insemination	(x)	( )		( )	
C-section	(x)	( )		( )	

R. **Body Fluid, Tissue, and Device Collection.** List each body fluid, tissue, or device to be collected, and complete the table below to indicate the nature of the collection. Check the relevant Appendices in Item Y, below, and complete and attach them, as shown in the column headings.

Body Fluid, Tissue, or Device to be Collected	Collected AFTER Euthanasia	Collected BEFORE Euthanasia		
		Blood Collection Associated with Antibody Production (Appendix 2, "Antibody Production")	Collected as Part of a Surgical Procedure (Appendix 5, "Surgery")	Other Collection from Live Animals (Appendix 4, "Antemortem Specimen Collection")
Blood	( )	( )	( )	(x)
Semen	( )	( )	( )	(x)
	( )	( )	( )	( )

S. **Surgery.** Does this protocol include any surgical procedure(s)?

►(x) Surgery WILL BE PERFORMED on some or all animals on this protocol. Check "Appendix 5" in Item Y, below, and complete and attach Appendix 5, "Surgery".
►( ) NO animals on this protocol will undergo surgery.

T. **Endpoint criteria.** Describe the criteria that will be used to determine when animals will be removed from the protocol or euthanatized to prevent suffering. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these criteria. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved).

In addition, specify how often the animals will be weighed to be sure weight loss does not exceed 10%.

(NOTE TO REVIEWERS: This section is important and should be carefully reviewed to ensure humane and safe endpoint criteria. The VMO should spend particular attention to this section as well. Departures from the Guide should be noted as described in Appendix 9).

► **None of the experimental procedures should affect the health of the animals. If the animal becomes sick (bad breath or drooling, excessive drinking or urination, appetite change associated with weight loss or gain, change in activity level (e.g., lack of interest in doing things), stiffness or difficulty in rising or climbing stairs, sleeping more than normal, or other behavior or attitude changes, coughing, sneezing, excessive panting, or labored breathing, dry or itchy skin, sores, lumps, or shaking of the head, digestive upsets or change in bowel movements, dry, red, or cloudy eyes) and loses more than 10% of its body weight (checked weekly) or if condition cannot be improved by medication as recommended by the veterinarian then the animal will be euthanized. It's important to maintain the fertility of the breeding dogs, so when the animal passes the prime age for breeding the old breeding animal will be either transferred to a research protocol or euthanized.**

U. **Termination or removal from the protocol**. Complete each of the following that applies:

►( ) Some or all animals will NOT be euthanatized on this protocol. Describe the disposition of these animals. (Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these methods of disposition. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)
►

►( ) Some or all animals MAY be euthanatized as part of the planned studies. Complete the table below to describe the exact method(s) of euthanasia to be used. (Use Appendix 9 to document any departures from the standards in the *Guide* represented by these methods. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

Check	Method of Euthanasia		AVMA Classification

each method that may be used on this protocol	Method of Euthanasia	Species	AVMA Classification		
			Acceptable	Conditionally Acceptable	Unacceptable
	CO_2 from a compressed gas tank Duration of exposure after apparent clinical death▶ Method for verifying death▶ Secondary physical method▶				
(x)	Anesthetic overdose Agent▶**Fatal Plus** Dose▶**100 mg/kg** Route of administration▶**I.V.** Method for verifying death▶**Cessation of breathing and heart beat.**		(x)	( )	( )
	Decapitation under anesthesia Agent▶ Dose▶ Route of administration▶				
	Exsanguination under anesthesia Agent▶ Dose▶ Route of administration▶ Method for verifying death▶				
	Other (Describe) ▶ Method for verifying death▶				
	Other (Describe) ▶ Method for verifying death▶				

1. For each of the methods above that is designated as "Conditionally Acceptable" by the AVMA, describe how the conditions for acceptability will be met:

   ► (x) **N/A**
   ► ( ) **Justification:**

2. For each of the methods above that is designated as "Unacceptable" by the AVMA, give the scientific reason(s) that justify this deviation from the AVMA Guidelines:

   ► (x) **N/A**
   ► ( ) **Justification:**

3. Identify all research personnel who will perform euthanasia on animals on this protocol and describe their training and experience with the methods of euthanasia they are to use in the species indicated.

   ► ( ) **N/A**
   [redacted]

4. Instructions for the animal care staff in case an animal is found dead.
   a. Describe the disposition of the carcass, including any special safety instructions. If disposition is to be handled according to a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
   (NOTE TO REVIEWERS: The VMU has a specific SOP (The Biocontainment SOP). Normally, this SOP would be the one used in this section. Only if some other SPECIFIC alternative procedure is used would the other box be checked.

   ► (x) **According to Biocontainment SOP.**
   ► ( ) **Not according to Biocontainment SOP:**
   ►**Justification and description (must review first with VMO):**

   b. Describe how the PI's staff should be contacted.

   ► (x) Please contact a member of the PI's staff immediately. (Copy the lines below for each individual who may be contacted)
   Name► [redacted]
   Contact Information► [redacted]

   Name► [redacted]
   Contact Information► [redacted]

   ► ( ) There is no need to contact the PI's staff immediately. Describe the routine notification procedures that will be followed. If the routine notification procedures are described in a local SOP, enter "according to local SOP" and enter the information requested about the SOP into the table in Item Y.
   ►

   Name►
   Contact Information►

V. **Special Procedures.** List each special procedure (including special husbandry and other special procedures) that is a part of this protocol, and specify where the details of the procedure are documented. See ACORP Instructions, for examples.

(NOTE TO REVIEWERS: This is a confusing section to understand and may require some additional discussion and review as to what special procedures should be listed here—but may generally include things like diet/fasting, restraint, etc).

Name of Procedure	Identify Where the Details of the Procedure are Documented		
	SOP (title or ID number)*	Other Items in this ACORP -- specify the Item letter(s)	Appendix 6
		Items:	( )**
		Items:	( )**
		Items:	( )**
		Items:	( )**

*If any special procedure is detailed in a SOP, identify the SOP and enter the information requested about the SOP in the table in Item Y.

**If any special procedure is detailed in Appendix 6, check "Appendix 6" in Item Y, below, and complete and attach Appendix 6.

(Use Appendix 9 to document any "departures" from the standards in the *Guide* represented by these procedures. Consult the IACUC or the Attending Veterinarian for help in determining whether any "departures" are involved.)

W. **Consideration of Alternatives and Prevention of Unnecessary Duplication.** These are important to minimizing the harm/benefit to be derived from the work.

(NOTE TO REVIEWERS: This new section is slightly modified from previous ACORP and focuses on replacement/refinement/reduction).

1. Document the database searches conducted.
   List each of the potentially painful or distressing procedures included in this protocol.
   - ► ( ) **N/A**
   - ► (x) **Painful or distressing procedures:**
     - ► **Intra-uterine insemination, C-section**

   Then complete the table below to document how the database search(es) you conduct to answer Items W.2 through W.5 below address(es) each of the potentially painful or distressing procedures.

PI should run at least one search on the ALTBIB website for animal use alternatives. Please use the link http://toxnet.nlm.nih.gov/altbib.html

Name of the database	Date of search	Period of years covered by the search	Potentially painful or distressing procedures addressed	Key words and/or search strategy used	Indicate which mandate each search addressed			
					Replacement of animals (item W.2)	Reduction in numbers of animals used (item W.3)	Refinement to minimize pain or distress (item W.4)	Lack of unnecessary duplication (item W.5)
Pubmed with animal alternative	5/1/17	2000-present	Narcolepsy, cataplexy, hypocretin		(x)	(x)	( )	( )
Pubmed with animal alternative	5/1/17	2000-present	Dog, intra-uterine insemination		(x)	(x)	( )	( )
Pubmed with animal alternative	5/1/17	2000-present	Dog, C-section		(x)	(x)	(x)	( )
Pubmed	5/1/17	1966-present	Narcolepsy, cataplexy, hypocretin, peptide, lateral hypothalamus		( )	( )	( )	(x)
Pubmed	5/1/17	1966-present	Narcolepsy, computer model		(x)	(x)	( )	( )
Pubmed	5/1/17	1998-present	Narcolepsy AND (mouse OR rat) AND hypocretin AND (induced cataplexy)		(x)	(x)	( )	( )

2. Replacement. Describe the replacements that have been incorporated into this work, the replacements that have been considered but cannot be used, and the reason(s) that further replacements are not acceptable.

► Our literature search came up no computer or in vitro models available for the study of narcolepsy. We are contrasting the canine model with the human disease. The symptoms seen in the dog closely resemble human symptoms and are therefore useful for the development of treatments. The advantage of using the canine model over rat and mouse model is that hypocretin cells are intact (but not the hypocretin receptor Type 2) in canine narcoleptics. Part of the proposed research studies are designed to study the release of hypocretin from these

cells, which is not possible in the rat and mouse model of narcolepsy in which hypocretin cells are either absent from birth or die shortly afterwards. In addition, cataplexy is readily inducible by food or play in the dog model but not in the mouse or rat model, which makes the dog model closer to the human condition and very valuable for studying cataplexy.

3. Reduction. Describe how the number of animals to be used has been minimized in this protocol and explain why further reduction would disproportionately compromise the value of the data.

   ► **We will only keep enough breeders to maintain a small colony. Further reducing the number of the animals will break down the breeding program.**

4. Refinement. Describe the refinements that have been incorporated into this work and explain why no further refinements are feasible.

   ► **The procedures we employ here are the best techniques available today to increase our chances of successful breeding. We will adopt whatever new and improved procedures become available in the future to reduce the pain and distress our dogs may experience during the breeding process.**

5. Describe how it was determined that the proposed work does not unnecessarily duplicate work already documented in the literature.

   ► **Our narcoleptic dog colony is currently the only one exists in the US and possibly the whole world. The work we will do with these dogs will build on our previous work in this area.**

X. **Other Regulatory Considerations**.

1. **Controlled drugs**.

► ( ) **N/A (Go to Question 2).**
► (x) Complete the table below for each drug that is used in animals on this protocol and that is classified as a controlled substance by the DEA. See ACORP Instructions, for explanations about the information requested.

Controlled substances	Storage		Personnel Authorized to Access	Location for Use		Procurement	
	Double-locked	Not Double-locked*		VA Property	Not on VA Property	VA Pharmacy	Non-VA
Propofol	(x)	( )*	[redacted]	( )	(x)	( )	(x)
Sodium pentobarbital (Fatal Plus)	(x)	( )*		(x)	( )	(x)	( )
		( )*					

*For any controlled substance that will NOT be stored under double lock, with limited access, describe how it will be stored, and explain why this is necessary.

► ( ) **N/A**
► ( ) **Justification:**

a. Check each statement below that applies, to confirm that all controlled substances used on this protocol will be procured according to VA pharmacy policies:

►( ) **N/A**
►( ) Some controlled substances will used on VA property, and all of these will be obtained through the local VA pharmacy.
►( ) Some controlled substances will not be obtained through the local VA pharmacy, but none of these will be used on VA property. See the ACORP Instructions, for further information.
►(x) Other. Explain► **Fatal Plus will be obtained through VA Pharmacy and used on VA property. Propofol is obtained by the private clinic and used in the clinic.**

2. **Human patient care equipment or procedural areas**. Does this protocol involve use of any human patient care equipment or procedural areas?

►( ) Yes, some human patient care equipment or procedural area(s) will be used for the animal studies on this protocol. Check "Appendix 7" in Item Y, below, and complete and attach Appendix 7, "Use of Patient Procedural Areas for Animal Studies".
►(x) No human patient care equipment or procedural areas will be used for the animal studies on this protocol.

3. **Explosive agents**. Does this protocol involve use of any explosive agent?

►( ) Yes, some explosive agent(s) will be used on this protocol. Check "Appendix 3" and "Appendix 8" in Item Y, below, and complete and attach Appendix 8, "Use of Explosive Agent(s) within the Animal Facility or in Animals", as well as Appendix 3, "Biosafety".
►(x) No explosive agent(s) will be used as part of this protocol.

Y. **Summary of Attachments.** To assist the reviewers, summarize here which of the following apply to this ACORP.

Appendices. Indicate which of the Appendices are required and have been completed and attached to this protocol. Do not check off or attach any appendices that are not applicable to this ACORP.

►(x) Appendix 1, "Additional Local Information"
►( ) Appendix 2, "Antibody Production"
►(x) Appendix 3, "Biosafety"
►(x) Appendix 4, "Ante-mortem Specimen Collection"
►(x) Appendix 5, "Surgery"
►( ) Appendix 6, "Special Husbandry and Procedures"
►( ) Appendix 7, "Use of Patient Care Equipment or Areas for Animal Studies"
►( ) Appendix 8, "Use of Explosive Agent(s) within the VMU or in Animals"
►(x) Appendix 9, "Departures from "Must" and "Should" Standards in the *Guide*"
►( ) Appendix 10, "Overnight housing"

Standard Operating Procedures (SOPs). List in the table below, each of the SOPs referred to in this protocol, providing the information requested for each one. The approved SOPs must be included when the approved ACORP and Appendices are submitted for Just-in-Time processing before release of VA funding support.

(NOTE TO REVIEWERS: This section will have to be developed over time. Some of the information will be pre-populated).

Item	SOP		Approval Date
	Title	ID	
C.2.c	This needs to be pre-populated		
M.1			
M.2			
U.4.a			
U.4.b			
V			

Z. **Certifications.** Signatures are required here for any ACORP that is to be submitted to VA Central Office in support of an application for VA funding. Include the typed names and dated signatures as shown below for the Main Body of the ACORP and for each of the Appendices that apply to this protocol. Do NOT include signatures for, or attach, any appendices that do NOT apply.

1. **Main Body of the ACORP.**

   a. **Certification by Principal Investigator(s):**

   I certify that, to the best of my knowledge, the information provided in this ACORP is complete and accurate, and the work will be performed as described here and approved by the IACUC. I understand that IACUC approval must be renewed at least annually, and that the IACUC must perform a complete *de novo* review of the protocol at least every three years, if work is to continue without interruption. I understand further that I am responsible for providing the information required by the IACUC for these annual and triennial reviews, allowing sufficient time for the IACUC to perform the reviews before the renewal dates, and that I may be required to complete a newer version of the ACORP that requests additional information, at the time of each triennial review.

   I understand that further IACUC approval must be secured before any of the following may be implemented:

   - Use of additional animal species, numbers of animals, or numbers of procedures performed on individual animals;
   - Changing any procedure in any way that has the potential to increase the pain/distress category to which the animals should be assigned, or that might otherwise be considered a significant change from the approved protocol;
   - Performing any additional procedures not already described in this ACORP;
   - Use of any of these animals on other protocols, or by other investigators.

   I further certify that:

- No personnel will perform any animal procedures on this protocol until the IACUC has confirmed that they are adequately trained and qualified, enrolled in an acceptable Occupational Health and Safety Program, and meet all other criteria required by the IACUC. When new or additional personnel are to work with the animals on this protocol, I will provide this information to the IACUC for confirmation before they begin work;
- I will provide my after-hours contact information to the animal care staff for use in case of emergency.

Principal Investigator		Date
[redacted]	[redacted]	[redacted]

b. **Certification by IACUC Officials.**

We certify that:

- We, with the IACUC, have evaluated the care and use of animals described on this ACORP, in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards, PHS Policy, the *Guide for the Care and Use of Laboratory Animals*, and VA Policy;
- The IACUC has determined that the care and use of animals described in this ACORP is appropriate, and has therefore approved the protocol;
- The full text of any minority opinions is documented here as indicated below:

► ( ) No minority opinions were submitted by any IACUC participant for inclusion.

► ( ) Minority opinions submitted by IACUC participants are copied here
►

► ( ) Minority opinions submitted by IACUC participants are attached on separate pages labeled "IACUC Minority Opinion" (indicate the number of pages►)

Name of Attending Veterinarian (VMO or VMC)	Signature	Date
[redacted]		
Name of IACUC Chair	**Signature**	**Date**
[redacted]		

2. **Appendix 2. Antibody Production.** No signatures required.

3. **Appendix 3. Biosafety.**

a. **Certification by PI(s) and IACUC Officials:**

We certify that:

- Before any animal experiments involving hazardous agents (identified in Item 10.a of Appendix 3) are performed, SOPs designed to protect all research and animal facility staff as well as non-study animals will be developed and approved by the appropriate VA or affiliated university safety committee and by the IACUC;
- All personnel who might be exposed to the hazardous agents (identified in Item 10.a of Appendix 3) will be informed of possible risks and will be properly trained ahead of time to follow the SOPs to minimize the risks of exposure.

Principal Investigator	Signature	Date
[redacted]	[redacted]	[redacted]
Name of Institutional Veterinarian	Signature	Date
[redacted]		
Name of IACUC Chair	Signature	Date
[redacted]		

b. **Certification by Biosafety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "toxic", "infectious", "biological", or "contains recombinant nucleic acid";
- The use of each of the agents thus identified as "toxic", "infectious", or "biological", or "contains recombinant nucleic acid" is further documented as required in Items 4, 5, 6, and/or 8, as applicable, and in Item 10.a of Appendix 3;
- The use of each of these agents has been approved by the appropriate committee(s) or official(s), as shown in Item 10.a of Appendix 3.

Name of the Biosafety Officer, or of the Chair of the Research Safety or Biosafety Committee	Signature	Date

[redacted]		

c. **Certification by Radiation Safety Official.** I certify that:

- Each agent to be administered to animals on this protocol has been properly identified in Item 1 of Appendix 3 as to whether it is "radioactive";
- The use of each radioactive agent is further documented as required in Items 7 and 10.a of Appendix 3;
- The use of each radioactive agent has been approved by the appropriate committee(s), as shown in Item 10.a of Appendix 3.

Name of the Radiation Safety Officer, or of the Chair of the Radiation Safety or Isotope Committee	Signature	Date

4. **Appendix 4. Ante-mortem Specimen Collection.** No signatures required.

5. **Appendix 5. Surgery. Certification by the PI(s).** I certify that:

- To the best of my knowledge, the information provided in Appendix 5 of this ACORP is complete and accurate;
- The surgical procedures will be performed and the post-operative care (including administration of post-operative analgesics) will be provided as described;
- The spaces where any survival surgical procedures will be performed (listed in Item 4 of Appendix 5) are suitable for sterile/aseptic surgery;
- The names and contact information for research personnel to notify or consult in case of emergencies will be provided to the VMU supervisor and veterinary staff;
- Post-operative medical records will be maintained and readily available for the veterinary staff and the IACUC to refer to, and will include the following:
  - Identification of each animal such that care for individual animals can be documented.
  - Daily postoperative medical records for each animal, that include documentation of daily evaluation of overall health and descriptions of any complications noted, treatments provided, and removal of devices such as sutures, staples, or wound clips;

- Documentation of the administration of all medications and treatments given to the animals, including those given to reduce pain or stress.
- Daily records covering at least the period defined as "post-operative" by local policy.
- The signature or initials of the person making each entry.

Principal Investigator	PI Signature	Date
[redacted]	[redacted]	[redacted]

6. **Appendix 6. Special Husbandry and Procedures.** No signatures required.

7. **Appendix 7. Use of Patient Care Equipment or Areas for Animal Studies.**

   a. **Certification by the Principal Investigator(s).** I certify that, to the best of my knowledge, the information provided in Appendix 7 of this ACORP is complete and accurate, and the use of patient care equipment or areas for these animal studies will be as described.

Principal Investigator	PI Signature	Date

   b. **Certification by the officials responsible for the use of any human patient care equipment in animal procedural areas.** Each of the following must sign to indicate that they have granted approval for the human patient care equipment to be moved to the VMU or other animal procedural area to be used on animals and then returned to the human patient care area, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of the Manager of the Human Patient Care Equipment	Signature	Date

   c. **Certification by the officials responsible for the use of the equipment in human patient care areas for these animal studies.** Each of the following must sign to indicate that they have granted

approval for animals to be transported into human patient care areas for study or treatment, as described in Appendix 7. Leave this section blank, if not applicable.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of the Chair of the Clinical Executive Board, or the Service Chief responsible for the Patient Care Area and Equipment	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of Chief of Staff	Signature	Date
Name of Director or CEO of the Facility (Hospital or Clinic)	Signature	Date

8. **Appendix 8. Use of Explosive Agent(s) within the Animal Facility or in Animals.**

   a. **Certification by the Principal Investigator(s).**

   I certify that, to the best of my knowledge, the information provided in Appendix 8 of this Animal Component of Research Protocol (ACORP) is complete and accurate, and the use of explosive agents in these animal studies will be as described.

   I further certify that:

   - Procedures involving explosive agent(s) will be performed within a properly operating, ventilated safety hood;
   - All electrical equipment operating when explosive agent(s) are in use will be positioned and powered outside of the hood;

- Once the seal is broken on any containers of explosive agents, they will be kept in a safety hood throughout use, stored in an explosion-proof refrigerator or other approved storage area, and discarded properly once completely emptied;
- Proper procedures will be used for safe and appropriate disposal of items (including animal carcasses) that may contain residual traces of the explosive agent(s).

Principal Investigator	PI Signature	Date

b. **Certification by the officials responsible for overseeing the use of explosive agent(s) in this protocol.** Each of the following must sign to verify that they or the committee they represent have granted approval.

Name of IACUC Chair	Signature	Date
Name of Attending Veterinarian (VMO or VMC)	Signature	Date
Name of Safety/Biosafety Officer for the Facility	Signature	Date
Name of ACOS for R&D	Signature	Date
Name of VISN Regional Safety Officer	Signature	Date

9. **Appendix 9. Departures from "Must" and "Should" Standards in the *Guide*.** No signatures required.

10. **Appendix 10. Certification by Principal Investigator is on the Appendix.**

Last Name of [redacted]
Protocol No. Assigned by the IACUC 9902-002
Official Date of Approval [redacted]

ACORP Appendix 1
ADDITIONAL LOCAL INFORMATION
VERSION 4 V2 6/17/2015
(Required for all protocols)

(See ACORP App. 1 Instructions, for more detailed explanations of the information req[redacted]

Species covered by this Appendix: **Dog**

This protocol involves the following (check all that apply):

☒ Breeding ☐ Tumor Formation ☐ Hazardous agents used in animals
☐ Multiple survival surgery ☐ Food and/or Fluid Restriction
☐ Antibody/Ascites Formation ☐ Hazard to VMU Personnel
☐ Prolonged Restraint (> than 15 minutes) ☐ Tumor formation

a.VA project # **0016**	b. Protocol # **9902-002**	c. 3 year expiration date [redacted]
[redacted]		
f. PI e-m[redacted]		g. Species: **dog**
h. Protocol title: **Breeding Protocol for Normal and Narcoleptic Dogs**		
i. Contact nam[redacted]		
k.Contact name 2:		l. e-mail:
m. Lab phone: [redacted]	n. Alternate phone:	o. EMERGENCY PHONE # (Cell preferred)
p. Animals taken to lab? ☐ Yes ☒ No If yes, Bldg: and Room(s):		
q. Animals taken to lab and then returned to vivarium (*VMU return room only*) ☐ Yes ☒ No If yes, provide a scientific justification here:		
r. Animals housed in the lab for 12 or more consecutive hours? ☐ Yes ☒ No If yes, Bldg: and Room(s): and fill out part B below.		
s. Is wire-floored caging required? ☐ Yes ☒ No		
t. Do animals need to be exempted from the environmental enrichment program? ☐ Yes ☒ No If yes, provide a scientific justification here:		
u. Maximum allowable body weight loss (10% unless scientifically justified): **10%**		
v. Hazards used in animals (check all that apply): ☒ None ☐ Toxic ☐ Infectious ☐ Biological ☐ Radioactive ☐ Other (list):		
w. Will VMU personnel be exposed to any of these hazards? (This includes animals housed in labs since VMU staff check them, wash the cages, etc.) ☐ Yes ☒ No If yes, list which hazards:		
x. Body fluid, tissue and/or device collection? ☐ None ☐ Live ☐ Dead ☒ Both		
y. Surgery? ☐ None ☐ Minor ☒ Major ☐ Both ☐ Non-survival Multiple survival surgeries? ☒ Yes ☐ No If there are multiple survival surgeries, list surgery types: **Intra-uterine insemination and C-section**		

z. Anesthetics/analgesics used (excluding euthanasia)? ☒ Yes ☐ No If yes, list: **propofol, Isoflurane, lidocaine, buprenorphine**
aa. Euthanasia methods (must include anesthetic plus physical method unless scientifically justified): **Barbiturate overdose with Fatal Plus**
bb. All controlled substances used: **Propofol, Fatal Plus**
cc. List any other drugs from Appendix 5 (surgery appendix): **5% Dextrose saline**

Delegation of Authority: Complete this section for every employee in this study, starting with the PI, specifying which procedures each is allowed to perform. All should be listed in the ACORP main body. Everyone listed must also have current employment status (VA or WOC) and be up-to-date with all required training and medical clearances.

Please note: There must always be at least one person responsible for task codes A, D, and H.

Species : **Dog**

Last name, first name, degree(s):	Task codes (use the list below):
[redacted]	**D, E, F, G, H, I**
	A, C, D, E, F, G, H, I

Task codes

A = Routine daily care of animal B = Performs survival surgery C = Performs non-survival surgery D = Evaluates endpoint criteria E = Collects samples with anesthesia F = Collects samples without anesthesia G = Collects or works with samples postmortem H = Euthanizes animal subjects	I = Performs in vivo procedures other than sample collection or surgery, such as behavioral studies J = Other work with animals, please specify: K = PI with no animal contact. The PI must be listed in section E of the ACORP Main Body. If the PI does have animal contact, list them with the appropriate task codes. L = Non-PI with no animal contact. This person does not need to be listed in section E of the ACORP.

Last Name of P[redacted]
Protocol No. Assigned by the IACUC ► 9902-002
Official Date of Approval ►[redacted]

ACORP Appendix 5

SURGERY
VERSION 4 V2 6-17-2015

See ACORP App. 5 Instructions, for more detailed explanations of the information requested.

1. **Surgery Classification.** Complete the table below for each surgery included in this protocol, and indicate how it is classified (terminal, minor survival, major survival, one of multiple survival). See ACORP App. 5 Instructions, for details.

Surgery		Terminal	Survival		
#	Description (specify the species, if ACORP covers more than one)		Minor	Major	One of Multiple*
1	**Intra-uterine insemination**	( )	( )	(x)	(x)*
2	**C-section**	( )	( )	(x)	(x)*
3		( )	( )	( )	( )*
4		( )	( )	( )	( )*

*If survival surgery (including major surgeries and any minor surgeries that may induce substantial post-procedural pain or impairment) will be performed as part of this protocol in addition to any other such surgery (on this or another protocol) on the same individual animal, complete items 1.a and 1.b, below:

a. Provide a complete scientific justification for performing the multiple survival surgeries on an individual animal:
► Inbreeding is known to cause low fertility, high puppy mortality, and other adverse side effects on breeding. Due to the nature of the disease and generations of inbreeding in narcoleptic dog colony, breeding for narcoleptic dogs have becoming more and more difficult. Although we have achieved some success with intravaginal and transcervical inseminations in the past, we were only succeeded in producing 3 puppies in the last three few years following 3 attempts on 3 bitches. And, that's with the help of intra-uterine insemination in the last attempt. We also have complications during the last 3 births during which natural birth was first attempted but all ended up with C-section. We will only try intra-uterine insemination if all other procedures failed. C-section is an option for us to avoid complications that may develop during birth.

b. Give the interval(s) between successive surgeries, and the rationale for choosing the interval(s):
► If intra-uterine insemination is performed and C-section is decided as the method of delivery after pregnancy is confirmed then the interval between the two procedures will be the period of gestation, around 60 days. Bitches that received c-section will have at least a year of rest before another breeding attempt is made. The vet at the clinic will be consulted regarding the maximum number of surgeries suitable for each bitch. Normally, not more than 6 surgeries (intra-uterine and c-section together) will be performed on each bitch during her breeding years. For bitches that have more than 2 pregnancies fewer surgeries may be performed because of the wear and tear of the uterus.

2. **Description of Surgeries.** Describe each surgery listed in Item 1, providing enough detail to make it clear what the effects on the animal will be. (Pre-operative preparation, anesthesia, and post-operative recovery will be covered in items 5, 6, and 7, below.)

   Surgery 1 ▶ ▶ With the dam under general anesthesia and in dorsal recumbency, the ventral abdomen is clipped, and after routine surgical preparation a 4 - 6 cm incision is made midway between the pubis and the umbilicus, through the linea alba. The uterus is elevated through the incision, and the needle of the syringe containing the semen is inserted into the lumen of the uterine body at a 45° angle with the bevel of the needle up. The semen is slowly injected into the uterus. A saline moistened gauze is held over the injection site after the needle is withdrawn. After 1 min the gauze is removed, the uterus replaced into the abdomen and the wound closed using routine methodology. To avoid backflow of semen the bitch is positioned with its rear elevated as she recovers from anesthesia. Size 3-0 monofilament absorbable sutures are used to close linea alba and subcutaneous openings. For skin closure, size 2-0 non-absorbable nylon suture is used. An appositional, simple continuous suture pattern will be used to close linea, body wall and subcutaneous fascia. Skin opening is closed with intradermal suture pattern.

   Surgery 2 ▶ **With the dam under general anesthesia and positioned in dorsal recumbency, a ventral midline laparotomy is made from the umbilicus to just cranial to the pubis. One gravid uterine horn and the uterine body are exteriorized. The abdominal cavity is protected from spillage of uterine contents with moistened laparotomy pads placed under and around the uterus. A stab incision is made in the ventral aspect of the uterine body, and extended with scissors. The puppies are then "milked" out of the hysterotomy, from cranial to caudal. Each puppy is handed off to the recovery team as it is delivered. When all the puppies have been removed from each horn of the uterus and before closing the hysterotomy, any remaining placentas are removed. A local anesthetic is administered around the incision and the abdominal wall is closed. Size 3-0 monofilament absorbable sutures are used to close uterus, linea alba and subcutaneous openings. For skin closure, size 2-0 non-absorbable nylon suture is used. An inverting suture pattern is used to close the uterine incision. An appositional, simple continuous suture pattern will be used to close linea, body wall and subcutaneous fascia. Skin opening is closed with intradermal suture pattern.**

   Surgery 3 ▶

   Surgery 4 ▶

3. **Personnel. Complete the table below for each individual who will be involved in any of the surgeries on this protocol.**

Name	Surgery #(s) (see	Role in Surgery

Name	Surgery #(s) (see	Role in Surgery			
		Surgeon	Assistant	Manage Anesthesia	Other (describe)
[redacted]	1, 2	(x)	( )	( )	( )
	1, 2	( )	(x)	(x)	( )

4. **Location of surgery.** Complete the table below for each location where surgery on this protocol will be performed.

Building	Room Number	Surgery #(s) (see Item 1)	Type of Space		
			Dedicated Surgical Facility	Other Dedicated Surgical Space	Other Space not Dedicated to Surgery
[redacted]		1	(x)	( )*	( )*
		2	(x)	(*)	( )*
				( )*	( )*
				( )*	( )*

*For each space that is not in a dedicated surgical facility, provide the justification for using this space for surgery on this protocol
▶

5. **Pre-operative protocol.**

   a. **Pre-operative procedures.** Complete the table below for each pre-operative procedure that will be performed to prepare the animal(s) for surgery.

Surgery #(s) (see Item 1)	Fast (Specify Duration)	Withhold Water (Specify Duration)	Place Intravenous Catheter(s) (Specify Site(s))	Other – Describe
1	( ) --	( ) --	(x) -- **cephalic vein**	( ) --

2	( ) --	( ) --	(x) -- **cephalic vein**	( ) --
3	( ) --	( ) --	( ) --	( ) --
4	( ) --	( ) --	( ) --	( ) --

b. **Pre-operative medications.** Complete the table below. Include agent(s) for induction of anesthesia, as well as any other pre-treatments that will be administered prior to preparation of the surgical site on the animal.

Agent	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of administration (e.g., times/day)	Pre-operative period of treatment (e.g., immediate, or # of days)
Propofol	**1 & 2**	**4.0 mg/kg; 1.2 ml**	**I.V.**	**Once**	**immediate**

c. **Pre-operative preparation of the surgical site.** For each surgery, identify each surgical site on the animals, and describe how it will be prepared prior to surgery.

Surgery 1 ▶ **The ventral abdomen about 4 - 6 cm midway between the pubis and the umbilicus is shaved. The incision site will be disinfected with three alternate applications of Nolvasan surgical scrub and 70% alcohol and then covered with a sterilized towel.**

Surgery 2 ▶ **The ventral midline from the umbilicus to just cranial to the pubis will be shaved. The incision site will be disinfected with three alternate applications of Nolvasan surgical scrub and 70% alcohol and then covered with a sterilized towel.**

Surgery 3 ▶

Surgery 4 ▶

6. **Intra-operative management.**

a. **Intra-operative medications.** Complete the table below for each agent that will be administered to the animal during surgery.

NOTE: If saline is being administered, it must be warmed to body temperature first.

Agent	Paralytic*	Surgery #(s) (see Item 1)	Dose (mg/kg) & volume (ml)	Route of administration	Frequency of dosing
Isoflurane	( )*	**1 & 2**	**1.5-5%**	**inhalation**	**Continuous during surgery**

2% lidocane	( )*	**1 & 2**	**4-10 ml**	**S.C.**	**Once before incision**
5% Dextrose saline	( )*	**1 & 2**	**10 ml/kg/hr**	**I.V.**	**Continuous during surgery**

* For each agent shown above as a paralytic, explain why its use is necessary, and describe how the animals will be monitored to ensure that the depth of anesthesia is sufficient to prevent pain.
►

b. **Intra-operative physical support.** For each surgery, describe any physical support that will be provided for the animals during surgery (e.g., warming, cushioning, etc.).
► Water-circulating heating pads and towels will be used to keep the animal warm during surgery.

c. **Intra-operative monitoring.** Describe the methods that will be used to monitor and respond to changes in the state of anesthesia and the general well-being of the animal during surgery.
► The vital signs of the animal (temperature, blood pressure, heart rate, respiration and oxygen saturation level) will be monitored continuously during the procedure and recorded in the surgery records. Blood pressure, heart rate and oxygen saturation will be monitored with a SurgiVet pulse oximeter. Temperature will be measured every 15 min with a rectal probe. Respiratory rate will be counted visually every 15 min.

7. **Survival surgery considerations.** For each survival surgical procedure indicated in Item 1 and described in Item 2, complete Items 7.a. – 7.g.

a. Complete the table below for each survival surgery listed in Item 1, above.

Surgery # (see Item 1)	Survival Period	Measures for Maintaining Sterility							
		Sterile Instruments	Surgical Cap	Sterile Gloves	Surgical Scrub	Sterile Drapes	Sterile Gown	Face Mask	Other*
1	**Life long**	(x)	(x)	(x)	(x)	(x)	(x)	(x)	( )*
2	**Life long**	(x)	(x)	(x)	(x)	(x)	(x)	(x)	( )*
3									( )*
4									( )*

* Describe any "other" measures to be taken to maintain sterility during surgery.
►

b. For each surgery, describe the immediate post-operative support to be provided to the animals.

Surgery 1 ▶ **The animal will be placed in the recovery room on a heating pad after operation. Pulse oximeter will continuously monitor the animal's blood pressure, heart rate and oxygen saturation. Supplemental 5% dextrose saline will be given intravenously to the animal whenever is needed. The animal will remain in the recovery room until fully ambulatory.**

Surgery 2 ▶ **The animal will be placed in the recovery room on a heating pad after operation. Pulse oximeter will continuously monitor the animal's blood pressure, heart rate and oxygen saturation. Supplemental 5% dextrose saline will be given intravenously to the animal whenever is needed. The animal will remain in the recovery room until fully ambulatory.**

Surgery 3 ▶

Surgery 4 ▶

c. Post-operative analgesia. Complete the table below for each surgery listed in item 1, above.

Surgery # (see Item 1)	Agent*	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of Dosing (e.g., times/day)	Period of treatment (e.g. days)
1	**Buprenorphine**	**0.01 mg/kg; 1-1.2 ml**	**IM**	**Twice/day**	**3**
2	**Buprenorphine**	**0.01 mg/kg; 1-1.2 ml**	**IM**	**Twice/day**	**3**
3					
4					

*For each surgery for which NO post-operative analgesic will be provided, enter "none" in the "Agent" column, and explain here why this is justified:
▶

d. Other post-operative medications. Complete the following table to describe all other medications that will be administered as part of post-operative care.

Surgery # (see Item 1)	Medication	Dose (mg/kg) & Volume (ml)	Route of Administration	Frequency of dosing (e.g. times/day)	Period of treatment (e.g. days)
1					
2					
3					
4					

e. Post-operative monitoring. <u>After-hours contact information for the personnel listed must be provided to the veterinary staff for use in case of an emergency</u>.

(1) Immediate post-operative monitoring

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**Once/15 min**	**Until fully ambulatory**	[redacted]
2	**Once/15 min**	**Until fully ambulatory**	
3			
4			

(2) Post-operative monitoring after the immediate post-operative period

Surgery # (see Item 1)	Frequency of Monitoring	Duration at this Frequency	Name(s) of Responsible Individual(s)
1	**Twice/day**	**3 days**	[redacted]
2	**Twice/day**	**3 days**	
3			
4			

f. Post-operative consequences and complications.

(1) For each surgery, describe any common or expected post-operative consequences or complications that may arise and what will be done to address them.

Surgery 1 ▶ **We don't expect this procedure to produce complications. If it does, most likely it's the infection at the incision site. VMO will be consulted and appropriate treatment will be given.**

Surgery 2 ▶ **We don't expect this procedure to produce complications. If infection occurs, VMO will be consulted and appropriate treatment will be given.**

Surgery 3 ▶

Surgery 4 ▶

(2) List the criteria for euthanasia related specifically to post-operative complications:

Surgery 1 ▶ **If infection persists even with treatments recommended by the VMO and the animal loses more than 10% of the weight then the animal will be euthanized.**

Surgery 2 ▶ **If infection persists even with treatments recommended by the VMO and the animal loses more than 10% of the weight then the animal will be euthanized.**

Surgery 3 ▶

Surgery 4 ▶

(3) In case an emergency medical situation arises and none of the research personnel on the ACORP can be reached, identify any drugs or classes of drugs that should be avoided because of the scientific requirements of the project. (If the condition of the animal requires one of these drugs, the animal will be euthanatized instead.)

▶ **A member of our staff, preferably [redacted] should be contacted immediately. The carcass should be left as is for us to remove the brain later (with the animal being transferred post-mortem to an experimental protocol so that the brain can be removed as part of an experimental protocol. The brains of these dogs are extremely valuable to our studies.) If none of our staff can be contacted immediately, the head should be removed and refrigerated. The body can be frozen.**

g. Maintenance of post-surgical medical records. Complete the table below for each surgery, specifying where the records will held, and identifying at least one individual who will be assigned to maintain accurate, daily, written post-surgical medical records. Indicate whether the named individuals are research personnel involved in this project, or members of the veterinary staff.

Surgery # (see Item 1)	Location of Records	Name(s) of Individual(s) Responsible for Maintaining Written Records	Research Personnel	Veterinary Staff
1	[redacted]	[redacted]	(x)	( )
2			(x)	( )
3				
4				

8. **Certification.** The PI must sign the certification statement in Item Z.5 of the main body of the ACORP.

Secondary Review

PI	STATION	FUNDING SOURCE	APPLICATION TITLE
[redacted]	Greater Los Angeles, CA - 691	Veterans Affairs	Breeding Protocol for Normal and Narcoleptic Dogs (Protocol 2016-060556)

ACTION NEEDED BY IACUC
The IACUC must review the concerns listed below and decide what response is needed. This action must be documented in the IACUC minutes and the changes required by the IACUC must be incorporated into the ACORP(s) and the revised ACORP(s) must be forwarded to the CVMO for archiving.

In case of questions about this review, please contact Dr. [redacted], Assistant Chief Veterinary Medical Officer at [redacted] or (615) 574-8198.

REVIEWER FEEDBACK

ACORP Item number(s)	Comments/Concerns
ACORP (dog)	This ACORP describes how a small colony of narcoleptic and normal dogs will be maintained to support studies investigating the cause and treatment of human narcolepsy. The research staff has many years of experience in breeding and maintaining the dog colony. The advantages of the canine narcolepsy model as opposed to a rodent model were clearly stated. A few aspects of protocol should be clarified. The specific numbered comments provided below must be reviewed by the IACUC, to determine what responses are needed. These actions must be documented in the IACUC minutes, and the changes required by the IACUC must be incorporated into the ACORP and the revised ACORP provided to the CVMO for archiving.
Items C.1, C.2, and I	Item C.1 indicates the dog colony consists of 1-2 breeding pairs while item C.2.b states the colony is only 3 dogs. Item I lists 2 normal dogs (1 breeding pair) and 3 narcoleptic dogs (1 male and 2 females). Please reconcile for consistency.
Item X and Appendix 5	Appendix 5 indicates that buprenorphine will be administered. Buprenorphine is considered a control substance, please list this analgesic in item X.
Item Y	According to the check marks shown in this item, appendices 1, 3, 4, 5, and 9 were enclosed. Appendices 4 and 9 were not included in the file. Please reconcile by either correcting item Y or providing the missing appendices.
Appendix 5	The skin is closed with non-absorbable nylon suture, when will the sutures be removed?

(cont.)

(cont.)