### Investigator

<table>
<thead>
<tr>
<th>Last Name:</th>
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<td>First:</td>
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<td>Department:</td>
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<td>Phone / Fax:</td>
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<td>After hrs. #:</td>
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</tbody>
</table>

### Species (common names): Rhesus macaque

<table>
<thead>
<tr>
<th>Number:</th>
<th>Source:</th>
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<tbody>
<tr>
<td>7</td>
<td>CNPRC</td>
</tr>
</tbody>
</table>

### Project Title

**Genes responsible for progesterone-withdrawal bleeding.**

### Overnight housing location:

| CNPRC | Day use only: CNPRC |

### Animals will be maintained by:

- [X] Vivarium  
- [ ] Investigator  

**If investigator maintained, attach husbandry SOP's.**

### Procedures:

Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

All macaques will be **ovariectomized** and will be treated with estradiol (E₂) and progesterone (P) implants to induce an artificial menstrual cycle. The endometrium will be collected by hysterectomy after different duration of P treatment and after P withdrawal as described below.

### Special Husbandry Requirements:

Describe any special requirements your animals have with respect to **food, water, temperature, humidity, light cycles, caging type, bedding,** or any other conditions of husbandry.

**No special husbandry required.**

### Other instructions for animal care staff: (check applicable entries)

**Sick Animals**

- [X] Call Investigator
- [X] Call Investigator
- [ ] Clinician to treat
- [ ] Terminate
- [X] Necropsy

**Dead Animals**

- [X] Call Investigator
- [X] Save for Investigator
- [ ] Bag for disposal
- [X] Necropsy

**Pest Control**

- [ ] Call Investigator
- [X] OK to use pesticides
- [ ] No Pesticides in animal area

### Hazardous Materials (only if in the animal room):

- Infectious Agents?  
  - [ ] Yes  
  - [X] No  

- Radioisotopes?  
  - [ ] Yes  
  - [X] No  

- Chemical Carcinogens?  
  - [ ] Yes  
  - [X] No  

- Toxic Chemicals?  
  - [ ] Yes  
  - [X] No  

Agent(s):
Funding source: Mellon Foundation

Previously approved? [ ] Yes [X] No

Is the project already funded? [X] Yes [ ] No

Previous protocol number (if any): 

**What Veterinarian or veterinary clinic will provide care for your animals? (check one)**

- [X] Lab Animal Health Clinic (2-0514)
- [ ] VMTH Large Animal Field Service (2-0292)
- [X] California Primate Research Center (2-0447)
- [ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:

- **Veterinarian:**
- **Address:**
- **Day phone:**
- **Emergency phone:**
- **Email:**

*If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@ucdavis.edu) for current information about training and record keeping requirements.*

**Summary of Procedures:**

**a)** Briefly describe the overall intent of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Regular bleeding is a feature of the normal menstrual cycle in primates; however little is known about the cellular and molecular events leading to this physiological tissue sloughing, bleeding and regenerative repair processes so characteristic of the primate endometrium. Despite the fact that steroid hormones are responsible for normal and abnormal endometrial bleeding, the identity of locally operative factors in endometrial bleeding are largely unknown. Clinically, extensive bleeding from the endometrium results in a range of disorders that significantly reduce the quality of life for women and accounts for more than half of the clinical practice in gynecology. Moreover, breakthrough bleeding is the most important cause for the discontinuation of extremely effective steroid contraceptives. Thus, a deeper understanding of the causes of endometrial bleeding would favor improvements in women’s health and is critically needed. Therefore, the main objective of this study is to identify specific novel genes responsible for P-withdrawal bleeding in the primate endometrium.

In the normal cycle, the endometrium undergoes a P-dependent transformation that makes it sensitive to bleed after P withdrawal. Several factors including metalloproteinases (MMPs), have been implicated in the processes of bleeding and tissue shedding during menstruation. MMPs facilitate breakdown of the extracellular matrix and increase dramatically within the human and macaque endometrium around the time of menses. We have recently demonstrated that P withdrawal dramatically up-regulates the VEGF type II receptor (KDR) in the same upper zones of endometrial stroma that expresses MMPs and sheds off during menstruation, suggesting a potential VEGF-KDR link in the menstrual induction cascade (et al. 2000).

In a recent work (unpublished), we found that an estrogenized endometrium (14 days of E2 treatment, E-14P) treated for 2 days with P would not bleed on P withdrawal, but one treated for 3-4 days with P would bleed profusely when P was withdrawn. A corollary to this observation is that antiprogestins do not induce bleeding when administered to cycling women in the follicular phase or immediately after ovulation. Bleeding only occurs if antiprogestins are administered during the mid to late secretory phase. Thus, it is clear that P transforms the endometrium into a “bleeding-sensitive” state as early as day 3 of the luteal phase. To investigate, whether bleeding induced after only 3 days of P treatment (E+3P-2P) is identical in all respects to that seen at the end of the cycle (E+14P-2P), we examined expression of MMPs and KDR in these two stages of endometrium in artificially cycling rhesus macaques. We observed profuse bleeding, however with very little tissue sloughing, on P withdrawal after 3 days of P treatment (E+3P-2P) compared to P withdrawal after 14 days of P treatment (E+14P-2P). Surprisingly, we did not find expression of any of the MMPs (MMP-1, -2, and -3) or KDR in the upper zones of endometrium on P withdrawal after 3 days of P treatment (E+3P-2P). Therefore, it is clear that P withdrawal bleeding can occur in absence of the well characterized factors associated with tissue sloughing during menstruation such as MMPs. These results suggest involvement of other genes/factors in inducing P withdrawal bleeding in primates. New advances in
molecular technology provide an exciting opportunity to address such questions. We propose to use microarray technology to reveal the changes in mRNA expression that transforms the endometrium into a “bleeding-sensitive” state, and to identify specific factors responsible for inducing endometrial bleeding worthy of additional study and subsequent validation.

Therefore, experiments are proposed to identify genes that transforms the endometrium into a “bleeding-sensitive” state (E-14P vs. E+3P), and to identify specific candidate genes responsible for P withdrawal bleeding by comparing differentially expressed genes on P withdrawal after a minimal period (3 days) of P treatment (E+3P vs. E+3P-2P) with P withdrawal after 14 days of P treatment (E+14P vs. E+14P-2P). Differentially expressed genes would reveal the probable candidate genes responsible for P withdrawal bleeding.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

[ ] Monoclonal Antibody Production **  [ ] Food or water restriction  [ ] Special diets; food or water treatment.
[ ] Polyclonal Antibody Production **  [ ] Non-recovery surgical procedures  [ ] Induced illness, intoxication, or disease
[ ] LD 50 or ID50 studies.  [x] Survival surgical procedures  [ ] Death as an endpoint (see i below)
[x] catheters, blood collection, intubation  [x] Multiple survival surgery  [ ] Trapping, banding or marking wild animals
[ ] Prolonged restraint. (8 hrs+)
 [ ] Behavioral modification.
 [ ]
[x] Fasting prior to a procedure.  [ ] Aversive conditioning.

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

A total of fifteen endometrial samples at different time-points (n=3/time-point) as indicated above are required for this experiment. Endometrial tissue samples previously obtained from eight monkeys (pilot study) by the PI (Dr. Nayak) at the Oregon National Primate Research Center will also be used for this experiment. Thus, seven new spayed artificially cycling monkeys as described below will be used for this experiment.

All animals will be anesthetized with ketamine (10 mg/kg), intubated and placed on isoflurane anesthesia. The animals will be given atropine (0.04 mg/kg) and receive a standard ovariectomy by CNPRC surgical staff. Following surgical recovery all macaques will be treated sequentially with estradiol (E2) and progesterone (P) implants to create artificial menstrual cycles. All implants (both E2 and P) will be inserted and removed from the animals at various timepoints (described below) while animals are briefly immobilized with ketamine (10 mg/kg) for each procedure. All macaques will receive s.c. (subcutaneous) implants of 3-cm Silastic capsules of E2 by a 1cm skin incision in the suprascapular region to stimulate development of an artificial proliferative phase endometrium. After 14 days, a 6-cm Silastic capsule of P will be implanted s.c., and both implants will remain in place for 14 days to stimulate an artificial secretory phase endometrium. Then at the end of the artificial cycle, the P implants will be removed by 1 cm cutaneous (skin) incision to induce menstruation while the E2 implants remain in place. The uterus from two animals will be collected on day 14 after P withdrawal (E-14P). The P implants will be inserted again in the remaining five monkeys on day 14 of the proliferative phase, and the uterus will be removed from two animals each on days 3 (E+3P) and 14 (E+14P) of the secretory phase. In the remaining one monkey, the P implant will be removed after 3 days of P treatment, and uterus from this animal will be collected on days 2 after the removal of P implant (E+3P-2P). The uterus will be collected either by euthanizing the animals or by hysterectomy. If hysterectomy is used, the animals will be anesthetized with ketamine (10 mg/kg), intubated and placed on isoflurane anesthesia. The animals will be given atropine (0.04 mg/kg) and receive a standard hysterectomy by CNPRC surgical staff. The animals will be monitored for presence of vaginal bleeding by vaginal swabbing after P withdrawal. A total of four peripheral venous blood samples (5 ml each) will be collected from each animal during the entire experiment (first collection, one day after E2
implant; second collection, one day after P implant; third collection, one day after P implant removal; and fourth collection, during tissue (uterus) collection. Blood samples will be collected while animals are cage restrained for first three time-points and during hysterectomy surgery for fourth blood collection. If the uterus will be collected by hysterectomy, the animals will be released for use by other investigators.

In order to complete the objectives outlined in this study, it is necessary to have the animals ovariectomized as to mimic an artificial menstrual cycle. At the conclusion of the treatments, it will be necessary to perform a second surgery (hysterectomy), in order to have enough tissue to identify the novel genes responsible for P withdrawal bleeding in the primate endometrium.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 days (E_2) treatment (E-14P)</td>
<td>2*</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>14 days (E_2) plus 3 days P treatment (E+3P)</td>
<td>2*</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>14 days (E_2) plus 14 days (E_2) and P treatment (E+14P)</td>
<td>2*</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>14 days (E_2) plus 3 days P treatment and after 2 days of P withdrawal (E+3P-2P)</td>
<td>1*</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>14 days (E_2) plus 14 days (E_2) and P treatment and after 2 days of P withdrawal (E+14P-2P)</td>
<td>0*</td>
<td></td>
</tr>
</tbody>
</table>
As indicated in section “c”, endometrial tissue samples, one each from groups-1 to -3, two samples from group-4, and three samples from group-5, were obtained from eight different monkeys (pilot study) by the PI (Dr. Nayak) at the Oregon National Primate Research Center. These endometrial samples will also be used for this experiment.

### Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1        | Little or no discomfort or stress  
**Examples:** domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral. |
| 2        | Minor stress or pain of short duration  
**Examples:** cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress |
| 3        | Moderate to severe distress  
**Examples:** major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation |
| 4        | Severe pain near, at or above the pain tolerance threshold  
**Examples:** exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness. |

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

The aim of this study is to identify specific novel genes responsible for P-withdrawal bleeding in the primate endometrium. Rhesus macaques are essential for this work because they menstruate identically to women when P level declines at the end of the menstrual cycle and upon P withdrawal in artificially cycling animals. Endometrial physiology including regulation by steroid hormones and menstruation, have been studied more intensively in rhesus macaques than any other nonhuman primate species. The rhesus macaque endometrium differentiates into morphological zones that are very similar to those of women. Moreover, we would like to compare the results of this study with our previous findings in the rhesus macaque model.

The procedures and the number of animals (n=3) proposed in this study are the minimum number of animals required for statistical validity of the results. In a pilot study conducted at the Oregon National Primate Research Center, eight monkeys (n=1 inc each from groups 1-3, n=2 from group 4, and n=3 from group 5) were already used in this study. Therefore, it will only be necessary to have seven animals from the CNPRC. Please note that the actual dose group numbers listed in section “d” are for only the numbers of animals needed from the CNPRC. The total numbers of animals used from both primate centers will add up to 3 animals per dose group (n=15).

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNPRC Surgical Suite&lt;br&gt;</td>
<td>1316</td>
</tr>
</tbody>
</table>

Who will be the surgeon? **Primate Center Veterinarians**

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>For immobilization (once per ovariectomy, implant placement, implant removal and hysterectomy)</td>
</tr>
</tbody>
</table>
Isoflurane  Inhaled  To eff  Per Surgery (ovariectomy surgery and hysterectomy surgery)

| Oxymorphone | .15 | IM | Two days post op per surgery |

h) Neuromuscular blocking agents can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

We do not anticipate any potential adverse effects of the experiment except minor stress or pain after hysterectomy.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Postoperative analgesics will be administered.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure?  [ ] Yes  [X] No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that this use of animals is not unnecessarily duplicative of other studies.

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search?  10/29/02

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>1990-2003</td>
<td>Endometrium, menstruation, nonhuman primate, healing, progesterone withdrawal, rhesus macaque, laparoscopy, laparotomy, hysterectomy,</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

The literature searches described above provided evidence that abnormal uterine bleeding is a significant cause of suffering for women which accounts for more than half of the clinical practice in gynaecology. The study further confirmed that studies of endometrial vascular development and bleeding can only be done in an in vivo model. Steroid hormones are responsible for normal and abnormal endometrial bleeding, but the identity of locally operative factors in endometrial bleeding and healing are not known. Breakthrough bleeding is the most important cause for the discontinuation of extremely effective steroid contraceptives. Decline in circulating progesterone level leads to menstruation and sloughing of upper zones of endometrium at the end of the menstrual cycle. Menstruation only occurs in women and nonhuman primates. Angiogenesis in the endometrium and menstruation occur as a result of complex interaction of several cell types in the primate endometrium, even by recruiting immune cells from systemic circulation. There is also a zonal gradient in expression of different tissue factors in the endometrium from cells in the functionalis to basalis. Therefore, nonhuman primates are the only suitable experimental animal model for this work and no alternatives to the proposed research exist. There are no less painful and/or stressful alternatives methods for tissue collection, and for insertion and removal of silastic implants of estradiol and progesterone.

Has this study been previously conducted?  [ ] Yes  [x] No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

Euthanasia is not part of the experimental design, but will be at the discretion of a senior veterinarian.

l) Methods of euthanasia: Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60</td>
<td>IM</td>
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</table>

m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

To be returned to the CNPRC colony.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don’t need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

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<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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<tbody>
<tr>
<td>CNPRC SRA’s</td>
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Occupational Health Program:
Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:
Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the UC Davis Policy and Procedure Manual section 290-30 (Animal Use and Care). This project will be conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals, and the UC Davis Animal Welfare Assurance on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at http://ehs.ucdavis.edu/). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

Principal Investigator | Rank / Title | Date

** Conditions necessary for Committee Approval:

Final Disposition of this protocol:

_______ Approved

_______ Not Approved

_______ Withdrawn by Investigator

Date of Action: _____ / _____ / _____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

Campus Veterinarian | Date
Hi,

I have received and pre reviewed the following protocol which has been assigned accession number 10664 for future reference. I have attached a copy of the protocol for ease of making revisions.

For this protocol to be considered on the July 3rd committee agenda, please return the revised document to me on or before noon, Tuesday, June 24th.

If you have any questions, feel free to contact me via phone or email.

Thanks in advance,

Protocol 10664 ( )
1. On the first page, the following boxes were left blank. Please complete: Overnight housing location; day use location and who will maintain the animals.

2. In section e, you have justified 3 per group, but you have 1 animal in group 4. Please clarify the difference.

3. In section c, you describe multiple survival surgeries. Please justify the reason for conducting the number of surgeries for this study.

4. In section g, you have listed the dose of oxymorphone as .75 mg/kg when the CNPRC vet staff use 0.15 mg/kg. Please confer with the vet staff and correct this section as needed. Under oxymorphone, you list two days post op, but then go on to state "one regimen per two surgeries". Please expand to explain what you mean by this statement.