PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CNPRC

Investigator

Last Name: ____________________________
First: ____________________________
Middle: ____________________________
email: ____________________________
Department: ____________________________
Phone: ____________________________
Fax: ____________________________

Contact

Last Name: ____________________________
First: ____________________________
Middle: ____________________________
email: ____________________________
Department: ____________________________
Phone: ____________________________
Fax: ____________________________

Species (common names): ____________________________
Number: ____________________________
Source: ____________________________
Rhesus monkey 44 Primate Center
(dams+infants)

Project Title: Androgens and Intrauterine Environment in Polycystic Ovarian Syndrome

Overnight housing location: Primate Center
Day use only: ____________________________
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.

These studies focus on the association between fetal androgen exposure and adult ovarian disease. Studies will include ultrasound imaging, maternal, fetal, and infant treatments, and maternal, fetal, and infant blood sample collection at different time points during gestation and postnatally.

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.

None

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

Sick Animals
[x] Call Investigator  [ ] Clinician to treat  [ ] Terminate  [ ] Necropsy

Dead Animals
[x] Call Investigator  [ ] Save for Investigator  [ ] Bag for disposal  [ ] Necropsy

Pest Control
[x] Call Investigator  [ ] OK to use pesticides  [x] No Pesticides in animal area

Hazardous Materials (only if in the animal room):

Infectious Agents? [ ] Yes  [x] No  Agent(s):
Radioisotopes? [ ] Yes  [x] No  Agent(s):
Chemical Carcinogens? [ ] Yes  [x] No  Agent(s):
Toxic Chemicals? [ ] Yes  [x] No  Agent(s):
Is the project already funded? [X] Yes [ ] No
Funding Source: NIH
Previously approved? [ ] Yes [X] No
Previous protocol number (if any): [ ]

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

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

If you checked “Another Veterinarian”, please provide:

Veterinarian: [ ]
Address: [ ]

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.

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy compromising the health of reproductive-aged women. It is a large contributor towards early-onset, type II diabetes mellitus, obesity, atherosclerosis and endometrial cancer. Recent studies have revealed that hyperandrogenemia is the major reproductive phenotype in PCOS kindreds, this phenotype is linked with a marker on chromosome 19p in the region of the insulin receptor gene, and this marker is associated with a metabolic phenotype in women with PCOS and male siblings. Based on these and other findings in human and animal models, it is hypothesized that fetal exposure to androgens during critical developmental stages results in the reproductive phenotype and the pancreatic β-cell dysfunction characteristic of PCOS. This hypothesis will be tested in the monkey model. These translational studies will be the first parallel investigations of human and monkey fetuses at risk for PCOS to determine similarities in timing and degree of ovarian hyperandrogenism and luteinizing hormone (LH) hypersecretion. Such comparisons will be crucial to conclusively demonstrate that fetal abnormalities conferred by genotype in humans can be duplicated by experimentally altered phenotype in monkeys. In this project it is hypothesized that a monkey model of PCOS, in utero exposure to androgen excess, defines the fetal origin of PCOS. It is proposed that hyperandrogenism, the core functional disorder in women with PCOS, reprograms multiple fetal organ systems resulting in the hormonal and metabolic abnormalities associated with PCOS. This study is a component of a multi-institution Specialized Center of Research on Sex and Gender Factors Affecting Women's Health (entitled “Genes, Androgens, and Intrauterine Environment in PCOS”), and will elucidate the mechanism(s) associated with PCOS.

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 h below)
[ ] catheters, blood collection, intubation  [ ] Multiple survival surgery  [ ] Trapping, banding or marking wild animals
[ ] Prolonged restraint. (8 hrs+)
[ ] Behavioral modification.
[ ] 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.)*

22 gravid animals and their respective offspring will be included in these studies. Only female fetuses will be used. Gender will be determined using established PCR-based protocols for detection of the rhesus Y chromosome in maternal blood samples collected at ~30 days gestation (~1 ml from a femoral vessel, see below) or chorionic villi collected by ultrasound-guided chorionic villus sampling (~25 days gestation), using established techniques. Ultrasound examinations to confirm normal development and viability will be performed every 7-10 days from ~20 days gestation until term delivery (see below) under ketamine or telazol. At 40 days gestation and daily until 80 days gestation, dams will be administered once daily subcutaneous (SQ) injections of either 15 mg testosterone propionate (TP) in 100 µl oil (N=11 treated) or 100 µl oil only (N=11 controls), using an established injection protocol [et al., 1997; et al., 2000]. The dams will be administered TP or oil under ketamine on days when scheduled for ultrasound examinations or injected in their home cage using CNPRC standard procedures (squeeze mechanism). The timing and dose of TP will produce circulating levels of testosterone in female rhesus fetuses approximately equivalent to fetal males, and will produce female monkeys with the phenotype necessary for these studies, based on prior investigations [et al. J Endocrinol 174:1-5, 2002]. Dams, fetuses, and infants will have blood samples collected and undergo hormonal challenges and tolerance tests as follows, all using established protocols for rhesus monkeys:

- **Maternal blood sample collection**: On days 25±5, 40, 60, 80, 100, 120 and at the time of cesarean-section, each dam will have an ~5-8 ml blood sample collected from a femoral vessel while under ketamine (coordinated with ultrasound examinations). CBCs will be assessed three times during gestation (~25±5, 120, and at term), and the balance of the samples will be used for hormonal analysis.

- **Amniotic fluid collection**: At 60, 80, 100, and 120 days gestation an ~1 ml volume of amniotic fluid will be collected under ketamine using established ultrasound-guided techniques for hormonal analysis.

- **Fetal gonadotropin releasing hormone (GnRH) and glucagon tests**: At 120 and 140 days of gestation, each dam will be administered telazol and supplemented with ketamine as needed for fetal treatment and sampling procedures. Fetuses will be administered 5 µg GnRH IV (~0.03 ml via the portal vein) using established ultrasound-guided techniques after an initial fetal blood sample has been collected (~400 µl) using established techniques. Fetal blood samples (~400 µl) will be collected at 10, 20, 30, and 40 min post-GnRH injection (total blood sample collected ~2 ml), using established ultrasound-guided techniques. On day 140 of gestation, an injection of 20 µg glucagon will be administered IV to the fetus (portal vein; ~0.03 ml) and ~400 µl blood samples will be collected at 0, 5, 15, 25, 35 and 45 min post-glucagon infusion (total volume ~2.4 ml). These volumes are within acceptable limits for fetuses in these age groups, and, based on extensive experience, both the volumes and sampling time points have previously shown to not result in fetal compromise. Tolbutamide (20 mg/kg, IV, ~0.03 ml) will be infused at 20 min after the glucose infusion to stimulate the pancreatic β-cells directly at the sulfonylurea receptor.

- **Term delivery**: At term (160±2 days), all newborns will be delivered by cesarean-section. Amniotic fluid (~5-6 ml) and cord blood samples will be collected (~6-8 ml). Simian Apgar scores and morphometrics will be assessed at birth. Simian Apgar scores are evaluated at 1, 5, and 10 min of life. This is a scoring system similar to the human Apgar where respiratory effort, heart rate, muscle tone, color, state, and body temperature are recorded. A complete placental evaluation will also be performed. All infants will be placed in incubators and reared in the nursery for postnatal studies.

- **Infant human chorionic gonadotropin (hCG) stimulation of ovarian steroidogenesis**: At ~2 weeks postnatal age each infant will be injected intramuscularly (IM) with 200 IU recombinant hCG (~0.3 ml volume), and ~1 ml blood samples will be collected from a femoral vessel in hand-held infants at 0, 24, and 48 hrs post-hCG injection (total volume ~3 ml) for hormonal analysis. These volumes are within acceptable limits for animals in this age group.

- **Infant GnRH stimulation of pituitary LH**: At ~1 month and 1.5 months postnatal age, each infant will undergo a GnRH challenge (7 am at 1 month, 9 pm at 1.5 months); ~400 µl blood samples will be collected from a femoral vessel from hand-held infants at 0, 10, 20, 30 and 40 min following the 20 µg GnRH IV injection (peripheral
vessel) (total volume collected at 1 month and at 1.5 months ~2 ml; these volumes are within acceptable limits for animals in this age group).

- **Intravenous glucose tolerance test (ivGTT):** At 70 days postnatal age, infants will be fasted overnight then administered ketamine at ~7 am in preparation for an ivGTT. Each animal will be administered 0.5 g/kg glucose (a stimulator of insulin secretion) via peripheral venous administration then 20 mg/kg tolbutamide (a sulfonylurea receptor stimulator) at 20 min post glucose injection via the same route. Blood samples (1 ml) will be collected from a femoral vessel -2, +5, 15, 25, 35 and 45 min (total blood collected: 6 ml). All sampling volumes are within the guidelines for animals in this age group.

- At 3 months of age, infants will be administered ketamine then euthanized for tissue collection.

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>Maternal TP administration day 40-80 of gestation SQ once daily, maternal blood sample collection, amniotic fluid and fetal blood sample collection, fetal GnRH and glucagon challenge, cesarean-section, infant hCG, GnRH, and ivGTT challenge, infant blood collection, and infant euthanasia and tissue harvest</td>
<td>22 (11 dams + 11 offspring)</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Maternal vehicle (oil) administration day 40-80 of gestation SQ once daily, maternal blood sample collection, amniotic fluid and fetal blood sample collection, fetal GnRH and glucagon challenge, cesarean-section, infant hCG, GnRH, and ivGTT challenge, infant blood collection, and infant euthanasia and tissue harvest</td>
<td>22 (11 dams + 11 offspring)</td>
<td>3</td>
</tr>
</tbody>
</table>

### Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress</td>
</tr>
<tr>
<td></td>
<td><strong>Examples:</strong> 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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
</tr>
<tr>
<td></td>
<td><strong>Examples:</strong> 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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td><strong>Examples:</strong> 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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
</tr>
<tr>
<td></td>
<td><strong>Examples:</strong> 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.</td>
</tr>
</tbody>
</table>

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

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

Monkeys are the only appropriate model for these studies because of developmental similarities when compared to humans. Extensive studies have previously characterized the best methods for developing the rhesus phenotype. Based on our experience and prior studies conducted to develop this model, the numbers proposed are based on statistical power estimates. A threshold probability of 0.05 for statistical significance will be applied. Previous findings suggest a sample size of 11 dams per group will yield a power of approximately 0.80-0.98.
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 animal quarters</td>
<td>Surgery suite</td>
</tr>
</tbody>
</table>

Who will be the surgeon? CNPRC 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>dams: ~10x; infants: 2x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>dams ~3 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Isoflurane</td>
<td>to effect</td>
<td>inhal.</td>
<td>Cesarean-section, 1x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>Post-surgery for dams, 2-3x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Buprenorphine</td>
<td>0.01-0.03</td>
<td>IM</td>
<td>Post-surgery for dams</td>
</tr>
</tbody>
</table>

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 adverse effects in dams, fetuses, or infants based on our extensive experience and published information on anatomical and hormonal outcome of monkeys exposed to androgens prenatally under a similar protocol [et al., 2002]. No effects are anticipated in adult animals (dams) because they are fully differentiated, and the TP exposure is of short duration and at a dose that has not been shown to result in any significant findings. Minimal discomfort may be associated with blood sample collection and cesarean-section. All possible measures will be taken to minimize discomfort from these procedures. Analgesics will be administered for 2 days post-surgery. Although the ovarian and pancreatic changes associated with androgen exposure are the focus of these studies, androgen exposure during 40-80 days gestation can result in virilization of external genitalia. This anatomical change (ambiguous genitalia) does not alter physiologic function, thus no adverse effects are anticipated. This is a common finding in human and nonhuman primate female fetuses exposed to androgens during the critical period of external genital differentiation. The pancreatic changes that may occur will not result in physiologic compromise that will place the animals health at risk. Our goal in these studies is to explore the underlying mechanism(s) responsible for adult onset disease, and to correlate findings in monkeys with those obtained in human studies. Only through the studies proposed in monkeys will the underlying mechanism(s) for PCOS be revealed.

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

See comments above. There are no other adverse effects anticipated or procedures planned that would require administration of analgesics or anesthetics other than those described above.
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:

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

What was the date on which you conducted this search?  6/1/02

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>1980 to current</td>
<td>Fetus, animal models, androgen excess, polycystic ovarian disease, polycystic ovaries, metabolic disease, fetal onset of adult disease</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent</td>
<td>Fetus, animal models, androgen excess, polycystic ovarian disease, polycystic ovaries, metabolic disease, fetal onset of adult disease</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are none that would allow us to investigate the questions we propose to address. A primate model is essential to conclusively demonstrate that fetal abnormalities conferred by genotype in humans can be duplicated by experimentally altered phenotype in monkeys as a result of prenatal androgen excess. The studies proposed cannot ethically be conducted in humans and require in vivo studies in an animal model that simulates human development.

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.

The studies outlined above are novel and have not been conducted in the manner we propose. Prior investigations were performed in monkeys to develop this model and show that fetal exposure to androgens can result in ovarian hyperandrogenism, polycystic ovaries, anovulation, central adiposity, and reduced insulin secretion. However, these studies did not address the mechanistic relationship of these findings to PCOS nor the outcome in a side-by-side translational comparison with a defined human population. As stated above, this study is a component of a NIH-funded Specialized Center of Research on Sex and Gender Factors Affecting Women's Health (entitled "Genes, Androgens, and Intrauterine Environment in PCOS") and will help to elucidate the pathogenesis of PCOS and ultimately provide the potential for molecular diagnosis of this syndrome in humans.

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

Infants will be euthanized at 3 months of age. The dams will be returned to the breeding colony two weeks post-cesarean-section.

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>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

See comments above.
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.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
</tr>
</thead>
<tbody>
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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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Professor</td>
<td>8/30/02</td>
</tr>
</tbody>
</table>

CNPRC Director

Date

Committee Use Only Below

** 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.

<table>
<thead>
<tr>
<th>Campus Veterinarian</th>
<th>Date</th>
</tr>
</thead>
</table>