PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

EH&S USE ONLY

CNPRC

PROTOCOL: 10672
EXPIRES: 6/18/04

Investigator

Last Name: 
First: 
Middle: 
email: 
Department: 
Phone / Fax: 
After hrs. #: 

Contact

Last Name: 
First: 
Middle: 
email: 
Department: 

Species (common names): 
Number: 
Source: 

Rhesus Monkeys 6 CNPRC

Project Title
The Hormonal Response of Daily Estrogen Administration

Overnight housing location:: 
CNPRC Day use only : 
CNPRC

Animals will be maintained by: 
[ ] 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.

Three male and three female monkeys will receive vehicle SQ administration for 2 days, followed by 4 days of SQ estrogen administration (dose = 350ug/kg/day). Blood samples (5ml) will be collected all 6 days at 0, 3, 6, and 10 hours post-vehicle/estrogen administration to measure hormone levels.

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.

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
[ ] 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):
**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.

The goal of this pilot study is to determine the hormonal response to daily estrogen administration. This paradigm will be used to test a drug developed by Merck for improving post-menopausal symptom in women in a future study (not covered by this protocol).

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

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

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**Funding source:** Merck

**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)**

- [ ] 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: __________________________

Day phone: __________________________

Emergency phone: __________________________

Address: __________________________

Email: __________________________

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

Animals: 3 males and 3 females sexually mature rhesus monkeys will be studied.

General Feeding Protocol: The animals will receive their standard chow diet during the 6 days of study.

Estrogen/Saline Administration: Three male and three female Rhesus monkeys will receive SQ vehicle administration for 2 days, followed by 4 days of SQ estrogen administration (dose = 350ug/kg/day).

Timeline (in days) PROCEDURE
1-2 SQ vehicle administration w/ blood draws at 0,3,6 & 10 hr post injection
3-6 SQ Estrogen administration w/ blood draws at 0,3,6 and 10 hours post injection

Blood Collection: Blood (4.0 ml) will be collected each day of the 6-day study at 1,3,6 and 10 hours post injection in order to measure plasma hormone levels.

Blood samples will be drawn from a cephalic vein in awake animals using arm-pull technique.

All blood draw volumes will comply with the CNPRC blood draw guidelines.

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>2 day vehicle/4 day Estrogen</td>
<td>3 males</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>administration</td>
<td>3 females</td>
<td></td>
</tr>
</tbody>
</table>


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?

This is a pilot study conducted in order to provide preliminary data to be used in the design of a more comprehensive experiment examining the effects of estrogenic compounds in rhesus monkeys. We expect that n=3 females and n=3 males will be sufficient to show the normal response to estrogen in both sexes.

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

Building:  
Room:  
Who will be the surgeon?

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>
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</thead>
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</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 adverse effects on the animals.

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.

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?  6-10-03

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>
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<tbody>
<tr>
<td>PubMed</td>
<td>1965-2003</td>
<td>Rhesus primate, estrogen, menopause</td>
</tr>
<tr>
<td>ISI Web-of-Science</td>
<td>1975-2003</td>
<td>Rhesus primate, estrogen, menopause</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There is no alternative methodologies

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?

Animals will be euthanized at the discretion of the CNPRC vet staff.
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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<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>pentobarbital</td>
<td>60 mg/kg</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?

All animals will be returned to the CNPRC colony.
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>
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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

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.

Campus Veterinarian | Date
To: Animal Care and Use Administrative Advisory Committee  
c/o Office of the Campus Veterinarian

From:

RE: Amendment to Protocol # 10672

Proposed Changes:

a. We would like to increase the total number of animals from 6 to 8. We will study one additional female and one additional male.

b. We will administer a Selective Estrogen Receptor Modulator (SERM) compound developed by Merck Inc. The animals will be chair-trained prior to study. Vehicle (0.25% methylcellulose) and SERM will be administered via naso-gastric tube in chair-restrained or hand-restrained animals.

c. SERM will be administered for 3 days at a low dose (2.5mg/kg/day as 0.5ml/kg). Following a 4-day washout period SERM will be administered at a higher dose (25mg/kg/day as 0.5ml/kg).

d. The blood collection schedule is amended as shown below and there will be 2 additional collections (highlighted in grey, 2-4 ml). Volume of blood collected will be determined by each animal’s body weight and will comply with the CNPRC blood draw guidelines.

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Blood Draws</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Vehicle</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
<tr>
<td>Day 2</td>
<td>Vehicle</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
<tr>
<td>Day 3</td>
<td>SERM (2.5mg/kg BW)</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
<tr>
<td>Day 4</td>
<td>SERM (2.5mg/kg BW)</td>
<td>0 hr bloods</td>
</tr>
<tr>
<td>Day 5</td>
<td>SERM (2.5mg/kg BW)</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
<tr>
<td>Day 6</td>
<td>None - Washout</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>None - Washout</td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td>None - Washout</td>
<td></td>
</tr>
<tr>
<td>Day 9</td>
<td>None - Washout</td>
<td></td>
</tr>
<tr>
<td>Day 10</td>
<td>SERM (25mg/kg BW)</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
<tr>
<td>Day 11</td>
<td>SERM (25mg/kg BW)</td>
<td>0 hr bloods</td>
</tr>
<tr>
<td>Day 12</td>
<td>SERM (25mg/kg BW)</td>
<td>0,3,6,10hr post-treatment</td>
</tr>
</tbody>
</table>

Justification for amendment:

This pilot study will compare the responses to a Selective Estrogen Receptor Modulator compound at a low and high dose to the normal response to daily estrogen administration determined in a pilot study. The additional blood draws will allow for adequate profiling of response to the vehicle and during low and high dose administration of SERM. As the responses
to this compound may be less robust than the responses to estrogen, we have increased the number of monkeys to be studied by one for both sexes (n=4 females and n=4 males).

Potential adverse effects:

We do not anticipate adverse effects. In previous long-term studies conducted by Merck with this SERM compound no adverse effects have been observed in rodents or in monkeys at these doses.