PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

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

Species (common names): Number: Source:
Cynomolgus and Rhesus macaque 60 (total for 3 years) CRPRC colony

Project Title
Molecular Morphogenesis and Toxicity in Primate Embryos and Fetuses

Overnight housing location:: CRPRC 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.

This project requires developmentally-staged cynomolgus and rhesus monkey embryos and fetuses ranging in age from gestation day (GD) 20-165 (term). Some pregnant animals will receive a test compound; others will be untreated or vehicle controls. Embryos and fetuses will be removed by hysterotomy at variable periods during development for morphological and histological evaluation as well as analysis of specific cell types using flow cytometry (FACS) and enzyme-linked immunospot assays (ELISPOT).

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 Dead Animals Pest Control
[ x ] Call Investigator [ x ] Call Investigator [ ] Call Investigator
[ x ] Clinician to treat [ ] Save for Investigator [ x ] OK to use pesticides
[ ] Terminate [ ] Bag for disposal [ ] No Pesticides in animal area
[ ] Necropsy [ x ] Necropsy

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): 

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This study is a continuation of ongoing research directed towards the molecular aspects of brain, craniofacial, cardiovascular, and immune system development in the macaque, a well-established developmental and teratological model for humans. Using immunohistochemistry, in situ hybridization, and cell analysis techniques, our current goal is to characterize cellular processes (e.g., programmed cell death [apoptosis]) as well as regulatory proteins which mediate specific aspects of morphogenesis (e.g., cytokines in immune system development). Perturbation of these normal cellular and molecular events will be studied using compounds suspected of inducing embryotoxicity (e.g., immunomodulatory agents such as retinoids, corticosteroids, and cytokines).

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.
- 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.

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.)
Females will be bred with fertile males during midcycle and pregnancy will be detected via ultrasonography on GD 12 (+2). Follow-up ultrasound procedures will be done periodically to monitor in utero growth and to determine the date of embryo/fetal removal by hysterotomy. All ultrasound procedures will be carried out on lightly anesthetized animals (ketamine). Approximately one-half of the pregnant animals will be dosed with a compound during pregnancy. Information on each compound (e.g., retinoids, corticosteroids, cytokines) used as well as the details of treatment (dose, route, frequency, length of treatment) will be summarized in amendments to this protocol.

Breeding, pregnancy detection/monitoring via ultrasound, and hysterotomies for control females will be the same as that described for treated females. However, these pregnancies will be untreated or treated with a vehicle on the same schedule as the test compound.

Ten control pregnancies per year are required in order to obtain embryos/fetuses representing a range of stages during brain, craniofacial, cardiac, and immune system development (e.g., GD 25, 50, 60, 80, 100, 140). These embryos will be fixed (e.g., Bouins), processed, and serially sectioned for immunohistochemistry and/or in situ hybridization assays. The antibodies and molecular probes used in these assays will aid in the identification of apoptosis and DNA synthesis as well as regulatory proteins of interest.

Ten treated embryos per year are also required in order to obtain a sufficient number with developmental perturbations which can be evaluated using the same morphological/histological techniques described for control embryos.

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>Control</td>
<td>Ultrasound, vehicle treatment, hysterotomy</td>
<td>10/year</td>
<td>3</td>
</tr>
<tr>
<td>Treated</td>
<td>Ultrasound, drug treatment, hysterotomy</td>
<td>10/year</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>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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
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<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
</tr>
<tr>
<td></td>
<td>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</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 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?

Both the rhesus and cynomolgus monkeys are well-established developmental and teratological models for humans due to qualitative and quantitative similarities in organogenesis as well as responsiveness to known teratogens (e.g., retinoids). A sufficient number of control embryos/fetuses (i.e., 10/year) is required to account for variability in normal development. An equivalent number of treated embryos/fetuses is needed to obtain a sufficient number with developmental defects which can be evaluated using the qualitative and quantitative techniques described.

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>CRPRC</td>
<td>1316</td>
</tr>
</tbody>
</table>

Who will be the surgeon?

CRPRC Veterinary staff

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 and Cynomolgus Monkeys</td>
<td>Ketamine HCl</td>
<td>10</td>
<td>IM</td>
<td>1x/day</td>
</tr>
<tr>
<td>Atropine</td>
<td>0.04</td>
<td>IM</td>
<td>1x/day</td>
<td></td>
</tr>
<tr>
<td>Isofluorane</td>
<td>To effect</td>
<td>Inhalation</td>
<td>1x/day</td>
<td></td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>3x/day for 3 days post-op</td>
<td></td>
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</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)

Post-hysterotomy discomfort may occur. Based on prior studies at the CRPRC, treated and vehicle-control animals may experience mild transient weight loss and/or poor appetite during the treatment period. Daily monitoring of all animals will ensure maintenance of health and animal well-being. Additionally, fruit supplementation will be employed, as needed, to keep the animals well nourished during the treatment period. Any anticipated adverse effects associated with specific compounds will be detailed in the amendment to the protocol for that compound.

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.

Post-hysterotomy discomfort will be alleviated with oxymorphone.
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?  April, 2001

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>Medline Healthstar</td>
<td>1980 – 2001</td>
<td>Nonhuman primates, macaques, morphogenesis, development (immune system, brain, craniofacial, heart) teratogenicity, embryotoxicity, immunomodulatory, apoptosis, specific regulatory factors (eg., cytokines)</td>
</tr>
<tr>
<td>Primate Literature Database (University of Washington)</td>
<td>1980 - 2001</td>
<td>Rhesus and cynomolgus macaques, morphogenesis, development (immune system, brain, craniofacial, heart), teratogenicity, embryotoxicity, immunomodulatory, apoptosis, specific regulatory factors (eg., cytokines)</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are numerous published studies in various animal models on the regulation of normal and abnormal developmental processes using molecular probes. However, to our knowledge most of the relevant studies on this topic in nonhuman primates have been carried out at the Primate Center, UC Davis. These types of studies continue to be important and relevant to humans since work done to date suggests that developmental regulation of many organ systems is species specific. This is particularly true for the immune system where the fetal macaque appears to develop some immunocompetency during the 2nd trimester while maturation of the immune system in rodents occurs postnatally.

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 CRPRC 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 and Cynomolgus Monkeys</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60 mg/kg</td>
<td>IV</td>
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</table>

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

Animals will be returned to the CRPRC colony at the end of the experiment.
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>
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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:

|   |   |   |

|   |   |   |

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|   |   |   |

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.

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