PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

PROTOCOL: 10706
EXPIRES: 7/30/04

Species (common names): Mucaca mulatta (rhesus monkey)
Number: 6
Source: CRPRC

Project Title: Host Response during Pulmonary Nocardiosis in Non-human Primate.

Overnight housing location: CRPRC TB 184
Day use: [ ]

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.

The host response of rhesus monkey, intrabronchially instilled with Nocardia asteroides strain GUH-2, will be investigated. Nocardiae inoculum of $1.5 \times 10^7$ CFU's will be administered to animals. Findings are relevant to understanding host response to pulmonary disease.

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.

Temperature (°F): 70-76 humidity (%): 40-60%
Hours light/hours dark: 12/12
Caging: SOP Size: NIH
Filter tops: No
Cage changes/week: 1/2
Bedding: none
Water: regular
Diet: Purina monkey Chow/twice a day

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

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>[x] Call Investigator</td>
<td>[x] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[x] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[ ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[x] No Pesticides in animal area</td>
</tr>
<tr>
<td>[ ] Necropsy</td>
<td>[ ] Necropsy</td>
<td></td>
</tr>
</tbody>
</table>

Hazardous Materials (only if in the animal room):

Infectious Agents? [x] Yes [ ] No
Agent(s): Nocardia asteroides GUH-2

Radioisotopes? [ ] Yes [x] No
Agent(s): 

Chemical Carcinogens? [ ] Yes [x] No
Agent(s): 

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.

Nocardia asteroides is increasingly recognized as a pathogenic agent causing pulmonary disease in both immunocompromised and healthy humans. However, there are no documented cases of human to human transfers or from animal to human transfers by aerosol. In previous mouse studies, the laboratory has housed healthy animals with nocardiace infected animals. These experiments have shown no evidence of animal to animal transfer. Furthermore, the current facility houses sentinel mice to monitor the possibility of nocardial transfer by aerosolization. To date, no nocardiace has been detected in these mice.

The mammalian host response against this pathogen varies by species and changes in nature with time after infection. We have developed a murine model to study host responses following infection by nocardiace. Data from these studies suggest that subsets of T-lymphocytes mediate clearance of bacteria attenuate inflammation and orchestrate repair in the lung. We hypothesize that in monkeys these subsets of T-lymphocytes function similarly to mount a host response to nocardiace. A comparative analysis in animals of variable sensitivity and variable respiratory anatomy with similarities and differences to humans will allow more confident extrapolation. The proposed monkey study will provide important information on the human host response during pulmonary nocardiosis.

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
- [x] Multiple survival surgery
- [ ] Behavioral modification.
- [ ] Aversive conditioning.

- [ ] Special diets; food or water treatment.
- [x] 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.)

**Bacterial culture:**

*Nocardia asteroides* strain GUH-2 will be grown from a frozen stock. One hundred µl of frozen stock will be added to 50 ml of Brain Heart Infusion broth (BHI-b) and incubated at 37°C with mild agitation (150 RPM) for 16-19 hours prior to use. The optical density will be measured on a spectrophotometer at 580 nm and the inoculum will be diluted/concentrated to an inoculum of 6 x 10**7** colony forming units (CFU)/ml.

**Osmotic minipump implantation:**

One week before nocardial infection, animals will be implanted with Alzet minipumps containing CldU, which is released at a constant rate. The half life of minipumps is two weeks. This is an accepted method to measure cellular turnover without radioactive materials. Prior to implantation, monkeys will befasted for 8 hours. Six rhesus monkeys (6-12 kg) will be anesthetized with either Telazol (0.8mg/kg IM) or Diprivan (0.1-0.2mg/kg/min IV) as selected by campus veterinarians. Animals will be restrained for less than 4 hours under continuous observation. At the moment of deep anesthesia, a small incision is made into the skin of back. The subcutaneous space is dilated by blunt dissection and an Alzet minipump is inserted. The incision is closed with sutures. Since the duration of experiment is to last no longer than 1 week, the removal of sutures will not be necessary. Sutures will take a week to heal by then tissue from animals will already be processed and sampled.

To combat possible adverse effects (infections) by the implantation of osmotic minipumps, we will use normal procedures described by a campus veterinarian. The antibiotic, Cephazolin will be given for a few days post surgery as a prophylactic. If an infection does occur, the pump will be removed and animal will be treated with antibiotics usually Cephazolin administered locally but can also be administered systemically. Although Cephazolin is the suggested, in cases where this antibiotic is ineffective, the site of infection will be cultured and specific antibiotic sensitivity panel will be performed. From this, specific therapy will be used. Treatment, dose and route will be followed at the discretion of a primate center veterinarian.

**Nocardial infection:**

Six rhesus monkeys (6-12 kg) will be intrabronchially infected with approximately 1.5 x 10**7** CFU of GUH-2. Monkey will be anesthetized intramuscularly with either Telazol (0.8mg/kg IM) or Diprivan (0.1-0.2mg/kg/min IV) as selected by a primate center veterinarian. Anesthesia is considered adequate when animals are completely relaxed and palpebral reflex is absent. Monkeys will be restrained for less than 4 hours under continuous observation. Application of topical anesthesia (lidocaine 0.5-1%) will be used on vocal cords, larynx, and trachea through the bronchoscope suction channel in a volume of 1-2 ml to suppress coughing and minimize aerosolization of nocardiae. A flexible fiberoptic bronchoscope will be inserted into the right bronchus. Two hundred and fifty microliters of GUH-2 in BHI-b will instilled into the right lung. To serve as the control, the left lung of animals will be instilled with 250 µl of BHI-b.

At 3, 5, and 7 days post infection, 2 monkeys/timepoint will be euthanized with an intravenous bolus of pentobarbital (60 mg/kg). At necropsy, animals will be exsanguinated and lung tissue will be removed. Tissue will be cut into smaller pieces. One half of the tissue will be snap-frozen in liquid nitrogen to be analyzed by *in situ* immunofluorescence or quantitative real-time PCR cytokine and chemokine expression. Some pieces will be fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned and stained with hematoxylin/eosin for histology and morphometry. The remaining tissue will be used to immunophenotype the host response. Lymphocytes will be isolated from the tissue and with specific T-cell markers (CD3, CD4, CD8 and γδ TCR) subsets of T-cells will be characterized at the respective timepoints. We will further characterize these subsets by intracellular staining of chemokines and cytokines. The control lung (left lobes) will be tested with the same molecular biological, histological, morphological, and immunological assays.

**Personnel:**

Trained CRPRC veterinary staff will perform Alzet minipump implantation and bronchoscopic procedures. CRPRC staff will observe animals post-operatively. As stated above, there have been no documented cases of nocardial transfer from human to human or animal to human by aerosolization. The sentinel mouse program has supported this observation. However, safeguards are in place to protect personnel during inoculation and post inoculation. To minimize exposure, all personnel will don personal protective equipment. CRPRC staff will be required to wear lab coats (disposable clothing), latex gloves, protective eyewear, surgical masks and booties. PPE should be autoclaved and disposed appropriately. PPE is required at all times in areas where animals are housed.
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>Day 3</td>
<td>nocardial infection, bronchoscopy, Telazol or Diprivan, lidocaine</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 5</td>
<td>nocardial infection, bronchoscopy, Telazol or Diprivan, lidocaine</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Day 7</td>
<td>nocardial infection, bronchoscopy, Telazol or Diprivan, lidocaine</td>
<td>2</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 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?

1) *Mucaca mulatta* (rhesus monkey) was chosen because species is closely related to human pulmonary airway immune system.

2) In the past, at least six mice (3 experimental/3 control/timepoint) was necessary to isolate minimum numbers of cells to be analyzed. Since the lung tissue of monkey is larger than the normal mouse we feel that the numbers of monkeys proposed would yield adequate numbers of cells from the tissue. In addition, two monkeys/timepoint is the minimum number to analyze biological variations in the specie as well as inherent variations in tissue processing. Because we are proposing to infect only the right lung, each monkey serves as its own control with a normal left lung. GUH-2 has shown to specifically attach to cells in the lung, we anticipate that most nocardiae will adhere and grow in a localized site (right lung) such that the left lung will not be affected.

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>1310</td>
</tr>
</tbody>
</table>

Who will be the surgeon? CRPRC 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>Mucaca mulatta</td>
<td>Telazol</td>
<td>0.8 mg/kg</td>
<td>intramuscular</td>
<td>pre-infection/once</td>
</tr>
<tr>
<td>Mucaca mulatta</td>
<td>lidocaine</td>
<td>0.5%-1%</td>
<td>topical</td>
<td>infection/as needed</td>
</tr>
<tr>
<td>Mucaca mulatta</td>
<td>Dipvian</td>
<td>0.1-0.2 mg/kg</td>
<td>intravenous</td>
<td>at specified timepoint/once</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?

No

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

Appearance of relaxed state and absence of the palpebral reflex.

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

Animals reject bronchoscope or exhibit coughing after introduction of bronchoscope.

**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)

Following administration of anesthetics, animals will experience adverse effects at level 2. The nocardial infection in mice and humans causes a mild-necrotizing pneumonia. In some cases, abcesses may form. Symptoms may include lethargy and hampered movement. In these cases, the whole lung was affected. In the proposed protocol, only half the lung will be inoculated with nocardiae while the other half of the lung remains healthy and fully functional. In this scenario, we do no anticipate that the animals will exhibit symptoms of disease as described by the literature.

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.

The inoculum proposed should be sublethal. Because this is a proposed pilot experiment, there is no way to know exactly what inoculum constitutes a sublethal dose without experimentation. However, we are proposing an inoculum that is pro-rated based on a sublethal dose for mice. Our lab has extensive experience with mice and has introduced the bacteria by many methods including intranasal and intravenous infection. From these studies we have enumerated an inoculum size that provides challenge to the animals but is sublethal. Therefore, the proposed dose for monkeys factors in the inoculum dose from mice and the relative size of the monkey lung compared to its murine counterpart. As stated earlier, the bacteria will be instilled in one half of the lung. The bacteria will adhere specifically to this portion of the lung. The monkey will have the other half of the lung healthy and intact. Considering that in the murine model the whole lung is affected and we are introducing the bacteria to only half the lung, we assume that this will be sublethal for the monkey. We expect animals to recover within a week without intervention. In such an event that the condition of the animals does not show signs of recovery, we will administer antibiotics such as sulfadiazene. Dosage, route and frequency will be at the discretion of the primate center veterinarian.

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_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? [5/30/03]

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>
</tr>
</thead>
<tbody>
<tr>
<td>Pubmed</td>
<td>ALL</td>
<td>rhesus, nocardia</td>
</tr>
<tr>
<td>Biosis</td>
<td>ALL</td>
<td>rhesus, nocardia</td>
</tr>
<tr>
<td>Current Contents</td>
<td>1993-</td>
<td>rhesus, nocardia</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

The intrabronchial infection will allow us to utilize the same animal to be an experimental and control subject. To this end, there are no alternative methodologies available.

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?

At the specified timepoints, animals are scheduled for euthanization. However, if animals do not appear to recover or show signs of recovery following infection by GUH-2 even after antibiotic treatment to alleviate distress, animals will be humanely euthanized. In addition, if any animals exhibit clinical signs of distress or discomfort that are untreatable, early euthanasia would be at the discretion of the attending 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>
</tr>
</thead>
<tbody>
<tr>
<td>Mucaca mulatta</td>
<td>intravenous</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?

No surplus
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>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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://ehs.ucdavis.edu/animal/health/](http://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at [http://ehs.ucdavis.edu/](http://ehs.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
ANIMAL ROOM SAFETY INFORMATION

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL #_10706__
EXPIRES: ________

RUA#: ________  BUA#: _0344_  CCA#: ________

Identity of Hazard: Nocardia asteroides

Investigator Last Name: ____________________________ First Name: ____________________________
Department: ____________________________ Phone: ____________________________
Email: ____________________________ Fax: ____________________________

Provide a short description of the agent:
Gram positive bacteria recognized as pathogenic agents causing pulmonary disease in both immunocompromised and healthy humans.

This agent / material is hazardous for: [ ] Humans only [ ] Animals only [x] Humans and Animals
For which Animal Species?

The agent can be spread by: [ ] Blood [ ] Feces/urine
[ ] Saliva/nasal droplets [x] Does not leave animal
[ ] Other:

Describe any human health risk associated with this agent:
Nocardial infection in humans causes a mild-necrotizing pneumonia. In some cases, abscesses may form. In rare cases, bacteria can be disseminated systemically.

The precautions checked below apply to this experiment:
[ ] The researcher or his/her technicians are responsible for the feeding and care of these animals.
[ ] The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.

[ ] Cage [ ] Stall [ ] Water Bottle [ ] Animal Carcasses
[ ] Bedding [ ] Other:

[x] Cages must be autoclaved before cleaning.
[x] Label cages and remove label after decontamination.
[x] Animal carcasses must be labeled and disposed of as follows:

[x] Incineration [ ] Biohazardous Waste Container
[ ] Bag and Autoclave [ ] EH&S will pick-up (2-1493).

[x] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows

[ ] Incineration [ ] Biohazardous Waste Container
[ ] Bag and Autoclave [ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required:
[x] The following personal protective equipment must be worn/used in the room:

[ ] Lab Coat/Coveralls [x] Shoe Covers/Booties
[ ] Disposable Gloves [ ] Head Cover
[ ] NIOSH Certified Dust Mask [ ] Disinfectant footbath
[ ] Eye Protection/Face Shield [ ]

[ ] Fitted Respirator Type:
[ ] Other: Describe: surgical mask

[x] Personal protective equipment must be removed before leaving the room.
[x] Personal protective equipment must be discarded or decontaminated at the end of the project
[x] Hands, arms, and face must be thoroughly washed upon leaving the room
[x] Full shower, including washing of hair, must be taken upon leaving the room.
[x] Decontaminate Room (inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room:
Pre review questions Protocol 10706

Hi,

I have received and pre reviewed the recently submitted protocol which has been assigned accession number 10706 for future reference. Since I have some questions, I have attached a copy of the protocol with the number embedded for ease of making revisions to the questions below.

For this protocol to be considered on the committee agenda of July 31, please forward your revised protocol to me on or before noon, Tuesday, July 22.

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

thanks in advance,

Protocol 10706 ( )
1. The funding source box on page 2 and the upper portion of the last page (animal room safety information sheet) were left blank. Please complete these box.

2. The protocol states that "this is a known pathogenic agent in healthy humans." It is also stated that the dose being given is a sublethal amount, however, this is a pilot study -- so how are lethal and sublethal doses known?

3. Will this agent be aerosolized if the animals cough -- during or after inoculation?

4. What precautions are being taken to protect people working with these animals -- both during inoculation and after?

5. In section c, you mention implanting Alzet mini pumps, but have not mentioned what happens once the incision is closed. When will the sutures be removed? Please clarify as well as describe when it is removed.

6. Since animals may exhibit some form of the disease, the category should be a 3 not a 2 in section d.

7. In section d, you discussed potential adverse effects associated with the nocardia, but what about possible problems with the mini pump implant? Please expand to address wound infection. What about antibiotics? what dose, route and frequency?

8. In section l, you pentobarbital at the dose of 120 mg/kg. However the CNPRC uses 60 mg/kg. Why is your dose different from that used by the CNPRC vet staff? Please clarify.