PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CNPRC

EH&S USE ONLY

PROTOCOL # _10301_

EXPIRES: ________

Investigator

Last Name:               Contact
First:                  Last Name:               Phone: work / home
Middle:                 First:                  work / home:
email:                  email:                  Departments:

Species (common names): Number: Source:
rhesus monkeys 59 CNPRC

Project Title: Stress-mitigating interventions in simian AIDS

Animals will be maintained by:  [X] Vivarium  [  ] Investigator

Overnight housing location:   [  ]CNPRC  Day use only :   [  ]CNPRC

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

Hazardous Materials (only if in the animal room):

Infectious Agents?  [X] Yes  [  ] No  Agent(s): simian immunodeficiency virus
Radiosotopes?  [X] Yes  [  ] No  Agent(s):
Chemical Carcinogens?  [X] Yes  [  ] No  Agent(s):
Toxic Chemicals?  [X] Yes  [  ] No  Agent(s):

Two studies will be performed, in which the negative effects of social stress in simian AIDS will be mitigated. In one study, animals experiencing stressful conditions will be switched to socially stable, non-stressful, groupings. In the second study, pharmacological manipulations will be employed which target the two physiological stress-response systems.
In the past decade, a growing body of evidence has accumulated showing that social stressors, bereavement, and the absence of social support experienced during the asymptomatic stage of HIV disease are associated with indicators of more rapid disease progression. Both in vivo and in vitro studies have suggested that the mechanisms mediating these effects involve the two major stress response systems, the sympathetic-adrenomedullary (SAM), and the hypothalamic-pituitary-adrenocortical (HPA). These results suggest that potentially useful adjunct therapies — behavioral or pharmacological — for HIV-infected individuals that target these physiological systems might lead to a reduction in HIV viral load and longer survival by altering stress-hormone concentrations, receptor numbers on immune cells, and cellular immune responses that are important in regulating viral expression.

Previous research using the SIV/rhesus macaque model of AIDS has demonstrated experimentally that psychosocial factors can affect the establishment of viral set-point and survival. The goal of the proposed studies is to determine the therapeutic efficacy of social manipulations and pharmacological substances that are aimed at reducing SAM and HPA activity, enhancing cellular immune function, and lowering viral load once a stable set-point has been established. For both studies, a number of viral, endocrine, functional immune, and histological measures will be obtained at regular intervals to test the hypotheses that interventions targeting these systems can affect SIV disease progression, and that the beneficial effects are mediated through stress response system effects on cell-mediated immunity. Our specific aims are to determine whether social stress and social stability affect SIV disease progression following establishment of viral set-point, and to determine whether nadolol, a beta-adrenergic receptor antagonist, and DHEA, an adrenal androgen associated with enhanced immune function, can ameliorate the effects of a social stressor on measures of SIV disease progression. This research will provide important new information on the interrelations of stress-response systems and immune function in the context of immunodeficiency disease, and will provide data demonstrating the efficacy of adjunct treatments that reduce the harmful physiological consequences of stress in AIDS.

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.
☐️ Survival surgical procedures
☐️ Death as an endpoint (see i 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.)

Three experiments are proposed.

In Experiment 1, 24 adult male rhesus monkeys will be relocated from their natal half-acre field cages to individual housing indoors. Animals will be formed into social groups (n=3 monkeys per group) for 100 min. per day, four days per week. Daily group formations will occur by transporting animals in individual transport boxes from their living cages to test rooms, where technicians will introduce the animals individually into our large social cages (10 ft. wide x 6 ft. deep x 7 ft. high). Following each 100-min. daily session, animals (who will have been pre-trained to exit the cages) will be returned in their individual transport boxes to their living cages. After 4 weeks of socialization sessions (Phase 1), all monkeys will be inoculated intravenously with SIVmac (1 ml, approx. 10$$^3$$ TCID50). Stable social conditions will continue for another 10 weeks (Phase 2), after which half of the animals will experience unstable social conditions for 5 weeks, while the remaining animals continue in stable social conditions (Phase 3). In unstable social conditions, 2-4 animals are placed together daily in each test cage, with membership in any particular group changing on a daily basis. During Phase 4, all animals will be returned to stable social conditions for 5 weeks. During Phase 5, half of the animals will again experience unstable social conditions for 5 weeks while the other half will remain in stable conditions, and in Phase 6, all animals will again be returned to stable social conditions for 5 weeks. All animals will then be euthanized. Table 1 shows the design:

<table>
<thead>
<tr>
<th></th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
<th>Phase 5</th>
<th>Phase 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinoc</td>
<td>4 wks</td>
<td>10 wks</td>
<td>5 wks</td>
<td>5 wks</td>
<td>5 wks</td>
<td>5 wks</td>
</tr>
<tr>
<td>Grp 1 (n=12) Stable</td>
<td>Stable</td>
<td>Unstable</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
<td></td>
</tr>
<tr>
<td>Grp 2 (n=12) Stable</td>
<td>Stable</td>
<td>Stable</td>
<td>Unstable</td>
<td>Stable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blood will be sampled from all monkeys twice at the end of each phase. One blood sample (drawn from an antecubital vein while the animals are in their living cages, 4 ml volume) will permit assessment of hematological and endocrine measures. The second blood sample (drawn under ketamine (10mg/kg) anesthesia, 15 ml volume) will be drawn during physical examinations, and cells from this blood draw will be cryopreserved for later immune and viral assay. The two blood samples will be drawn on two separate occasions during the last week of each phase; blood volumes are well within CNPRC guidelines for adult male rhesus monkeys. Urine will also be collected from each monkey at the end of each phase according to standard CNPRC procedures. This will involve placement of a pan to collect urine beneath each cage prior to lights out, and retrieval of the pan and urine soon after lights on the next morning. Urine will be assayed for concentrations of catecholamine metabolites. During physical examinations, mucosal swab samples will be taken by rubbing a sterile Dacron swab inside the lower lip. Saliva samples will be used for assessment of cytomegalovirus viral copy number. Once inoculated with SIV, animals will receive physical examinations at 2.5 week intervals (i.e. twice in every 5-week period).

Experiment 2 will involve 8 monkeys. This will be a short-term study (approx. 1 month in duration) to determine the proper doses for the drugs to be administered to the animals in Experiment 3. We will use adult male rhesus monkeys from the CNPRC colony, and return them to the colony at the end of the study. Nadolol, a beta adrenergic receptor antagonist, will be administered orally for 3-day periods at 40, 100, and 200 mg per day. Animals will be monitored for signs of excessive sympathetic nervous system
suppression (including signs of distress, sedation, changes in activity, feeding, etc.).

After 48 hrs. at each dose, blood pressure and heart rate will be assessed following immobilization with ketamine (10mg/kg). Washout periods of seven days will be included between doses for all animals. A second dose-finding study will be conducted with the animals to find the proper dose for our second drug, DHEA (dehydroepiandrosterone), which is a naturally-occurring androgen of adrenal origin. We will select doses that give us a 6-8 fold increase in plasma levels of DHEA-S, the sulfated form of DHEA. In humans, doses typically range from 50-500 mg per day, and no side effects have been found for doses ranging up to 2500 mg/day. As with the nadolol study, we will monitor behavior in the animals to determine whether there are any adverse effects, and final doses of each drug will be chosen for Experiment 3 that produce the desired effects with minimal side effects.

Experiment 3 will use 27 adult male rhesus monkeys. As with Experiment 1, all animals will experience 4 weeks of stable social conditions (Phase 1), after which the animals will be inoculated intravenously with SIVmac (1 ml, approx. 10^3 TCID50), and stable conditions will continue for 10 weeks (Phase 2). At this point, all animals will be switched to unstable social conditions, which were described in Experiment 1 above. One third of the animals will receive placebo (sugar pill), one-third will receive nadolol, and one-third will receive DHEA, in doses determined during Experiment 2. All drugs will be administered orally, either mixed with food, or in a fruit-drink solution. Unstable conditions with drug administration will occur for 5 weeks (Phase 3). During Phase 4, all animals will receive the placebo administration (this serves as a drug washout period) for 5 weeks, after which drugs are administered for another 5-week phase (Phase 5). Finally, a second 5-week washout period will complete the study (Phase 6), after which all animals will be euthanized. The table below shows the experimental design schematically:

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase 1</td>
</tr>
<tr>
<td>Preinoc</td>
<td>Post-inoc</td>
</tr>
<tr>
<td>4 wks</td>
<td>10 wks</td>
</tr>
<tr>
<td>Grp 1 (n=9)</td>
<td>Stable</td>
</tr>
<tr>
<td>Grp 2 (n=9)</td>
<td>Stable</td>
</tr>
<tr>
<td>Grp 3 (n=9)</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Blood, urine, and saliva sampling will occur at the end of each phase, exactly as described for Experiment 1. As in Experiment 1, once animals are inoculated with SIV, animals will receive physical examinations at 2.5 wk. intervals.

We note that we have trained animals to enter and exit the cages, and have formed social groups of adult male rhesus macaques in the manner we are proposing as part of other approved research projects. We have 10 years of experience with these procedures, and our safety procedures (described in section i. below) have resulted in virtually no injurious aggression requiring veterinary intervention.

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>social group formation, SIV inoculation, physical examination, venipuncture, ketamine immobilization</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>nadolol and DHEA administration, physical examination, ketamine immobilization</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

University of California, Davis
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Categories of invasiveness

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

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

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

The SIV/rhesus macaque model of AIDS is the best animal model available. Number of animals in each group was determined using power analysis. In Experiment 1, using the cross-over design described above, power to detect a 0.6-log difference in viral load, using a 1-tailed test (higher viral load in unstable, compared to stable, animals) with effect size d=1.1, is 0.70. In Experiment 2, power to detect a 1.2 log differences is 0.72. For Experiment 2, our short-term dose-finding study, we will use 4 animals for the nadolol condition and 4 animals for the DHEA condition. Given the extent of individual variation, 4 animals is the smallest number we can use to determine consistency of our results and to ensure that side effects are minimal (or nonexistent) for the drug doses we are proposing.

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 minor discomfort that is more than slight or momentary. Postoperative analgesics are not to be given, justify the practice under part (f) 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>10mg/kg</td>
<td>i.m.</td>
<td>for physical examinations (at the end of each phase)</td>
</tr>
<tr>
<td>rhesus</td>
<td>nadolol*</td>
<td>40-200 mg/day</td>
<td>oral</td>
<td>daily during phases 3 and 5 of experiment 3.</td>
</tr>
<tr>
<td>rhesus</td>
<td>DHEA**</td>
<td>50-500 mg/day</td>
<td>oral</td>
<td>daily during phases 3 and 5 of experiment 3.</td>
</tr>
</tbody>
</table>

*note exact doses for Experiment 3 will be determined from data obtained in Experiment 2
b) 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?

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)

1. Animals in unstable social conditions may experience mild stress during group formations. Following SIV inoculation, animals will develop signs of immuno deficiency disease, which can include neurological deficits, weight loss, and/or opportunistic infections.
2. Animals may experience side effects from the nadolol or DHEA administered during Experiment 3.

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.

1. Stress will be minimized by having the animals in the unstable social conditions for the smallest amount of time necessary to establish the necessary risk of study. Stress is minimized by a) having canine teeth clipped/blunted (depending on age); b) having animals under observation at all time by trained behavioral observers; c) spraying the animals with a jet of water from a hose to break up escalating bouts of aggression. These three procedures have been virtually 100% effective in our 10 years of conducting such research with adult male rhesus monkeys. 2. Animals will receive specific and supportive therapy for any signs of SIV disease they may exhibit. Animals will be euthanized when the attending veterinarian determines the animal is no longer responding to treatment. 3. Dosage of nadolol and DHEA for Experiment 3 will be chosen based on a previous dose-finding study (Experiment 2), in which animals will be closely monitored by behavioral observers, animal health technicians, and veterinary staff, to insure that any side effects will be minimal.

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? 9/9/02

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>mid-60s - present</td>
<td>stress AND AIDS AND viral load</td>
</tr>
<tr>
<td>psycinfo</td>
<td>1872 - present</td>
<td>stress AND AIDS AND viral load</td>
</tr>
<tr>
<td>current contents</td>
<td>1993 - present</td>
<td>stress AND AIDS AND viral load</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

In vitro work has been conducted examining how stress-related hormones can affect viral expression. The next step is to determine whether those suggestions (which have informed the proposed experiments) are effective in whole animals, prior to the start of any clinical trials. There are no alternatives to use of live animals for examining how social or pharmacological treatments affect stress-induced immunodeficiency disease progression.

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.

Disposition of animals:

At what point in the study, if any, will the animals be euthanized?

All animals will be euthanized at 30 weeks after SIV inoculation, or sooner if the attending veterinarian determines that animals are no longer responding to specific and supportive therapy for their immunodeficiency. (According to CNPRC “Criteria for Euthanasia of SIV Animals”)

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>intravenous</td>
</tr>
</tbody>
</table>

Surplus animals:

What will you do with any animals not euthanized at the conclusion of the project?

Subjects in Experiment 2 will be returned to the CNPRC colony.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don’t need to include the staff of the vivarium in which your animals will be housed.

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

<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>
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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 adequately 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), 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 # 10301
EXPIRES: ________

RUA#: __________________ BUA#: 0528 CCA#: __________

Identity of Hazard: simian immunodeficiency virus

Investigator Last Name: __________________________________ Department: __________________
First Name: __________________________________________ Phone: __________________

Provide a short description of the agent:

SIV is a primate lentivirus that is genetically similar to HIV and causes fatal immunodeficiency in infected rhesus macaques. SIV can infect humans but it is unknown whether it causes human disease.

This agent / material is hazardous for: [ ] Humans only [ ] Animals only [x] Humans and Animals

For which Animal Species? rhesus monkeys

The agent can be spread by: [x] Blood [ ] Feces/urine
[ ] Saliva/nasal droplets [ ] Does not leave animal
[ ] Other: mucosal (eye, mouth, nose, genital)

Describe any human health risk associated with this agent:

SIV can infect humans; thus it is possible that SIV could cause a fatal, AIDS-like disease in humans. Infectious virus and SIV antibodies have been detected in SIV-infected humans, but there have been no reports of disease in such people.

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.
[ ] Label cages and remove label after decontamination.
[ ] Animal carcase must be labeled and disposed of as follows:
[ ] 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  [x] 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
[x] Disposable Gloves  [x] Head Cover
[ ] NIOSH Certified Dust Mask  [ ] Disinfectant footbath
[x] Eye Protection/Face Shield  [x] tyvek disposable gowns/coveralls
[ ] Fitted Respirator Type: __________________

[x] Personal protective equipment must be removed before leaving the room.
[ ] Personal protective equipment must be discarded or decontaminated at the end of the project.
[ ] 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:

Biosafety (BSL) 2+ precautions must be used at all times.