

**PROTOCOL FOR ANIMAL USE AND CARE**Email to: [campusvet@ucdavis.edu](mailto:campusvet@ucdavis.edu)**CNPRC**

EH&amp;S USE ONLY

**PROTOCOL: 10487****EXPIRES: 3/13/04**

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

Species (common names):	Number:	Source:
Macaca mulatta	18	CNPRC

<b>Project Title</b>	Estrogen Loss and Serotonin Function in Primates		
Overnight housing location::	CNPRC	Day use:	LBNL and UCDMC
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> 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.

Ovariectomized monkeys will be transported to LBNL for SPECT imaging studies and returned to the CNPRC at the end of the day. Animals will be injected with a radiotracer (containing iodine 123) at the CNPRC and transported several hours later. Animals will be anesthetized for the SPECT studies and allowed to wake up before returning to the 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.

Twelve of eighteen monkeys will receive either tamoxifen or estrogen in their daily food.

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

Sick Animals	Dead Animals	Pest Control
<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input checked="" type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

**Hazardous Materials** (only if in the animal room):

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	Iodine 123
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NIH	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

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

<input type="checkbox"/>	Lab Animal Health Clinic ( 2-0514 )	<input checked="" type="checkbox"/>	California Primate Research Center ( 2-0447 )
<input type="checkbox"/>	VMTH Large Animal Field Service ( 2-0292 )	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

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

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

The proposed studies will evaluate the effects of estrogen loss on serotonin function in a primate model of menopause. A great deal of interest has been focused on the effects of estrogen on various measures of brain function because of evidence that estrogen may have neuroprotective effects and may reduce the risk of Alzheimer's disease in postmenopausal women. A large amount of rodent data shows effects of estrogen and estrogen loss on various neurotransmitter systems, including the serotonin system. Effects of estrogen on serotonin function have been implicated in depression, especially depression following the onset of menopause. While it has been suggested that antidepressant effects of estrogen are mediated by serotonin, relatively few studies have been performed in primates to evaluate the effects of estrogen on serotonin function, particularly with regard to short-term versus long-term effects. Such studies are needed in order to better understand the mechanisms of estrogen-mediated effects and could have implications for the management of depression and the use of estrogen replacement therapy in postmenopausal women. We propose to use SPECT and **the serotonin tracer, iodine-123-5-iodo-6-nitroquipazine (INQUIP)**, to evaluate serotonin function in ovariectomized female macaques, before and after short- and long-term treatment with the estrogen antagonist, tamoxifen, estrogen replacement therapy, or vehicle (control). Tamoxifen is proposed as a means of evaluating dose-response effects of estrogen on serotonin function since it is known to have estrogen-antagonistic effects in at least some brain regions. Monkeys will receive SPECT scans before treatment and following short-term (1 month) or long-term (1 year) treatment. We hypothesize that estrogen replacement therapy will be associated with increased serotonin function and tamoxifen with decreased serotonin function, with the vehicle treated animals intermediate to the other two groups. We expect these effects to be reversed when treatment is stopped. The proposed studies will not only determine the short-term and long-term effects of estrogen loss on serotonin function, but may have implications for women undergoing tamoxifen treatment or estrogen replacement therapy.

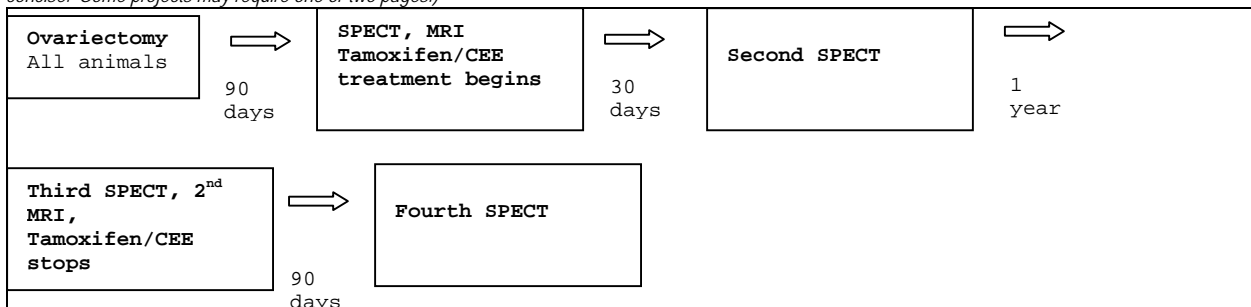
**b) Procedures employed in this project:**

Please check the appropriate boxes if any of these procedures will be employed in your project:

- |   |  |   |
|---|--|---|
| <input type="checkbox"/> Monoclonal Antibody Production **                  | <input type="checkbox"/> Food or water restriction               | <input checked="" type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production **                  | <input type="checkbox"/> Non-recovery surgical procedures        | <input type="checkbox"/> Induced illness, intoxication, or disease          |
| <input type="checkbox"/> LD 50 or ID50 studies.                             | <input checked="" type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below)                 |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery               | <input type="checkbox"/> Trapping, banding or marking wild animals          |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+)                      | <input type="checkbox"/> Behavioral modification.                | <input type="checkbox"/>  |
| <input checked="" type="checkbox"/> Fasting prior to a procedure.           | <input type="checkbox"/> Aversive conditioning.                  | <input type="checkbox"/>  |

\*\* 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.)



A total of 18 ovariectomized female *Macaca mulatta* will be studied with SPECT and the serotonin radiotracer, iodine-123-5-iodo-6-nitroquipazine (INQUIP) in order to evaluate both short and long-term effects of treatment with tamoxifen, ERT, or vehicle. SPECT scans will be performed 3 months after ovariectomy, following 30 days of treatment, and following 12 months of treatment. An additional SPECT scan will be performed 90 days after treatment has ended in order to determine the reversibility of any effects. In addition, each monkey will receive an MRI at the Medical Center in Sacramento. The MRI will be co-registered with the SPECT scans for the identification of brain regions. **This involves matching the 3D anatomical data from the MRI with the 3D SPECT data in order to identify anatomical regions on the SPECT scans.** Monkeys will receive an MRI at baseline and following 12 months of treatment in order to evaluate the effect of treatment on hippocampal volume. Below is the timeline for the procedures.

*Ovariectomy*

All animals will undergo bilateral ovariectomy in the CNPRC centralized surgical suite. Each animal will be anesthetized with 10 mg/kg ketamine IM and given 0.04 mg/kg atropine SQ. The animals will then be placed on isoflurane anesthesia to effect and positioned in dorsal recumbency. A ventral caudal midline abdominal incision will then be made to visualize the body of the uterus and both ovaries. Ovarian vessels and the fallopian tubes will be isolated, ligated, and severed. The abdominal wall will then be closed in three layers with two layers of 2/0 absorbable suture and the final subcuticular layer with 3/0 absorbable suture. The animals will then be recovered in the CNPRC surgical recovery unit and given two days of post-operative analgesia, 0.15 mg/kg oxymorphone IM three times daily.

*Tamoxifen Treatment*

Six monkeys will be treated with tamoxifen. Tamoxifen will be administered

daily in food for 12 months. The dose was computed to be equivalent to the usual dose given to women by adjusting doses for metabolic rate. Each monkey will receive 1.3 mg/kg per day which corresponds to a woman's dose of 20 mg/d. **Tamoxifen treatment will begin approximately 3 months after ovariectomy, following the first SPECT study.**

#### *Estrogen Treatment*

Six monkeys will be treated daily with conjugated equine estrogens (CEEs). The dose will be computed to be equivalent to a human dose of 0.625 mg/day. CEEs will be administered in food for 12 months. **CEE treatment will begin approximately 3 months after ovariectomy, following the first SPECT study.**

**Both tamoxifen and estrogen will be administered in one of the following ways:**

- 1. Mixed with tang and hand fed (w/syringe)**
- 2. Mixed with Boost and hand fed (w/syringe)**
- 3. Mixed in baby food and hand fed (on treat board)**
- 4. Frozen into tang-cubes and hand fed**

#### *Control Treatment*

**The control animals will be fed the same diet as the treatment animals but no drug treatment will be administered.**

In the event that an animal refuses to ingest the tamoxifen or estrogen in food, oral gastric tubing will be used. An 8-french feeding tube (approx 60-80cm) will be attached to a syringe filled with fresh water. The animal will be hand caught, removed from the cage, and restrained while an SRA snakes the tube through the nose, down the esophagus and into the stomach. At that point we will aspirate gently to verify we are in the stomach, remove the water, attach the dose syringe, dose the animal, flush it with the water and remove the tube. The entire procedure takes about 1-3 minutes and after a few days the animals habituate to the procedure.

#### *MR Studies*

Animals will be transported to the University of California Medical Center Imaging Facility in Sacramento in **a Primate Center van accompanied by a veterinarian and a Staff Research Associate or Animal Health Technician.** Data will be acquired on a 1.5T GE Signa Horizon LX Echospeed system. The animal will be anesthetized with ketamine (15 mg/kg), and medetomidine (30 mcg/kg) and placed in a plastic stereotaxic frame. After a conventional sagittal scout scan (TR/TE 500/12 ms, flip angle 90°), a volumetric T<sub>1</sub>-weighted SPGR (spoiled gradient recall acquisition in the steady state) scan will be acquired. The SPGR scan will contain 124 contiguous transaxial slices of 1-mm thickness (with no interslice gap) through the entire brain. Following the imaging study the animal will be recovered from anesthesia and returned to the CNPRC.

#### *SPECT Studies*

SPECT (**Single Photon Emission Computed Tomography**) studies will be performed at the Lawrence Berkeley National Laboratory (LBNL) using a General Electric Millennium VG dual-headed gamma camera with the Hawkeye CT system. Animals will be transported from the CNPRC to LBNL on the day of the study and returned at the end of the day. A veterinarian and a trained technician will accompany the animals to LBNL and will be present for the entire scanning session. Since the optimal imaging time with INQUIP is approximately 17 hours following injection, the tracer, which will be produced at LBNL, will be transported to the CNPRC the day before the study for injection and the animals will be transported to LBNL the following day

for imaging.

Approximately 6 hours before to tracer injection, animals will be treated with 15 mg/kg of potassium iodide (PI) via gastric tube to block uptake of the tracer into the thyroid gland. **Animals will be injected with telazol (8 mg/kg IM) for anesthesia** and 7-10 mCi of the tracer will be injected intravenously. **Animals will be transported to LBNL in a Primate Center van accompanied by a veterinarian and a Staff Research Associate or Animal Health Technician.** On the day of the study, after transportation to LBNL, animals will again be anesthetized with ketamine, given 0.04 mg/kg of atropine (0.26 mg), intubated and ventilated with isoflurane. A blood sample will be taken at this time and later assayed (at the CNPRC) for serum E<sub>2</sub> levels using radioimmunoassay methods previously describe. Animals will then be positioned in the stereotaxic frame and placed in the scanner. Imaging will begin and continue for 1 hour. A CT scan will be performed in order to correct the images for attenuation. **This corrects for the differences photon absorption and scatter due to differences that the radioactivity (photons) must travel from different parts of the brain to reach the detectors in the SPECT scanner.** Below is a timeline for these procedures.

**Pre-treatment**

**4.5 hours later**

**1.5 hours later**

**(6 hours after PI pre-treatment)**

**17 hours later**

15 mg/kg potassium iodide (PI)

10 mg/kg ketamine for anesthesia

Tracer injection

SPECT scanning will begin at LBNL

**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.

Group	Procedures / Drugs	Number of Animals	Category
1	Ovariectomy, daily dosing with tamoxifen, SPECT (4 times), MRI (2 times).	6	3
2	Ovariectomy, daily dosing with estrogen, SPECT (4 times), MRI (2 times)	6	3
3	Ovariectomy, no drug treatment, SPECT (4 times), MRI (2 times) ( <b>control group</b> )	6	3

## Categories of invasiveness

Category	Description
1	Little or no discomfort or stress <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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 <b>Examples:</b> 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?

We have selected rhesus monkeys primarily because we require an animal with a brain suitable for study with a scanner with 7mm resolution. The goal of the proposed studies is to evaluate effects of estrogen on brain function that are likely to be applicable to humans. While similar studies have been performed in rodents, there is some evidence for species differences and the results of these studies do not necessarily apply to primates. We have chosen to study monkeys rather than humans because of the many confounding variables that are difficult to control in human studies of this type. In addition, given the current controversy with respect to the use of estrogen replacement therapy, studies involving estrogen treatment are not feasible in humans. We have designed these studies based upon our previous experience with neuroimaging studies in monkeys. Based on these earlier studies, as well as other studies evaluating the effects of hormones on serotonergic measures, 6 animals per group, for a total of 18 animals, is appropriate.

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.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus	Ketamine	10-15	IM	immobilization
	Isoflurane	Inhaled	to effect	Surgery & scans
	Oxymorphone	.15	IM	two days post op
	Atropine	.04	SQ	Surgery & scans
	Telazol	8 mg/kg	IM	Injection of tracer

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)

Animals may experience slight distress upon induction of anesthesia and discomfort following ovariectomy. Animals may also develop a post-operative infection from ovariectomy. There should be no pain or distress during imaging as the subjects will be anesthetized. There is also a potential for hypothermia. Side effects associated with tamoxifen include symptoms of menopause such as hot flashes, and nausea or vomiting. Less common side effects are blurred vision, confusion, weakness and sleepiness. Tamoxifen is also associated with an increased risk of uterine cancer. Side effects associated with estrogen include nausea, bloating and headaches and usually resolve within a few weeks. Less common side effects include liver and gall bladder disease and hypertension.

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.

All animals will receive a minimum of two days post-op analgesia. Animals will be observed daily for post-op complication and treated by the CNPRC veterinary staff. During the scans, animals will be placed on and covered with heating pads. Animals will also be monitored by pulse oximetry and a temperature probe.

*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  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](http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm) (email: [jawelsh@ucdavis.edu](mailto:jawelsh@ucdavis.edu))*

*or [http://www.vetmed.ucdavis.edu/Animal\\_Alternatives/main.htm](http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm) (email: [mwood@ucdavis.edu](mailto:mwood@ucdavis.edu))*

What was the date on which you conducted this search?

2 / 5 / 03

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PUBMED	1994-2003	INQUIP, serotonin, SERT, estrogen
Current Contents	"	"

What were your findings with respect to alternative methodologies?

Alternative methodologies require death as an endpoint.

Has this study been previously conducted?

Yes  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?

NA

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.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus	Overdose	Pentobarbital	60	IM

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





**ANIMAL ROOM SAFETY INFORMATION**

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

**PROTOCOL # 10487****EXPIRES:** \_\_\_\_\_RUA#: 1071

BUA#: \_\_\_\_\_

CCA#: \_\_\_\_\_

Identity of Hazard: I123

Investigator Last Name: \_\_\_\_\_ Department: \_\_\_\_\_

First Name: \_\_\_\_\_ Phone: \_\_\_\_\_

Email: \_\_\_\_\_ Fax: \_\_\_\_\_

**Provide a short description of the agent:**I123 used during scanning for SPECT studies. Animals transported from LBL to CNPRC

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

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

**Describe any human health risk associated with this agent:**Animals injected with I123 are considered radioactive for 24-48 hours. Urine and Feces will contain trace amounts and should be avoided.**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:
- Cages must be autoclaved before cleaning.  
 Label cages and remove label after decontamination.  
 Animal carcasses must be labeled and disposed of as follows:  
 Incineration  Biohazardous Waste Container  
 Bag and Autoclave  EH&S will pick-up (2-1493).  
 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:**

- The following personal protective equipment must be worn/used in the room:  
 Lab Coat/Coveralls  Shoe Covers/Booties  
 Disposable Gloves  Head Cover  
 NIOSH Certified Dust Mask  Disinfectant footbath  
 Eye Protection/Face Shield   
 Fitted Respirator Type:  
 Other: Describe:
- Personal protective equipment must be removed before leaving the room.  
 Personal protective equipment must be discarded or decontaminated at the end of the project  
 Hands, arms, and face must be thoroughly washed upon leaving the room  
 Full shower, including washing of hair, must be taken upon leaving the room.  
 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:**All waste must be collected and handles as wet and dry radioactive waste according to procedures in RUA approval