PROTOCOL FOR ANIMAL USE AND CARE
Handwritten forms are not accepted

CNPRC

Investigator

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>email:</th>
<th>Department:</th>
<th>Phone / Fax:</th>
<th>After hrs. #:</th>
</tr>
</thead>
</table>

Contact

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<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>email:</th>
<th>Department:</th>
<th>Phone:</th>
<th>After hrs. #:</th>
</tr>
</thead>
</table>

Species (common names): Rhesus monkey
Number: 10
Source: CRPRC

Project Title: Effect of elevated copper intake on infant health II.

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.

Weaned infants (6 mo) will be fed regular monkey chow and followed through 12 mo. Animals will receive an oral supplement (3 ml, daily) of a copper solution or water. Copper absorption will be assessed using 67Cu at 9 and 12 mo, urinary copper excretion will be assessed at 9 and 12 mo, liver biopsies will be taken at 9 and 12 mo and blood will be drawn monthly from 6 to 12 mo.

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.

Animals will be fed normal monkey chow and supplemented (daily) with a copper sulfate solution or equal amount of water.

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

Hazardous Materials (only if in the animal room):

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Radioisotopes?</th>
<th>Chemical Carcinogens?</th>
<th>Toxic Chemicals?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Yes  [ x ] No</td>
<td>[ x ] Yes  [ ] No</td>
<td>[ ] Yes [ X ] No</td>
<td>[ ] Yes  [ X ] No</td>
</tr>
</tbody>
</table>

Agent(s): 67Cu
Copper is an essential nutrient but can also be toxic at high dietary levels. Elevated exposure of copper is of concern in areas where industrial mining is conducted and where copper water pipes are used or where well-water pH is low. Furthermore, the presence of a polymorphism in a copper transport protein (Wilson mutation) may make a large percentage of the population more susceptible to copper toxicity. However, the range of safe dietary copper intake, particularly for infants and children, as well as the their ability to regulate copper absorption to protect themselves from copper toxicity, has not been adequately established. Unfortunately, biomarkers for determining elevated copper exposure are inadequate, as circulating copper and copper transport proteins are maintained across a wide range of copper intakes. We hypothesize that weaned infants have the ability to regulate gastrointestinal copper absorption and that this ability protects them from copper toxicity. In this project we propose to use juvenile rhesus monkeys to determine effects of elevated dietary copper during late infancy on copper absorption, liver patho-physiology, circulating liver enzymes and hematological parameters from 6 to 12 mo of age.

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.
- Special diets; food or water treatment.
- Induced illness, intoxication, or disease
- Death as an endpoint (see i below)
- Trapping, banding or marking wild animals
- 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.)

Animals will be fed standard monkey chow.

Group 1: copper-supplemented (6 mg copper as copper sulfate), given orally in water from a syringe (3 ml/d).

Group 2: control, 3 ml water/d, given orally from a syringe.

Weight and length measurements will be taken monthly from 6-12 mo.

Animals will be fasted for 4 hours prior to all blood draws. Blood will be drawn monthly form 6-12 mo.

Animals will be housed individually and a 24-hour urine collection will be performed at 9 and 12 mo.

At 9 and 12 months, animals will be fasted for 4 h followed by an oral-gastric gavage of 3 ml formula with 1 uCi 67Cu and counted in a whole body counter. Animals will be recounted 4 days later.

At 9 and 12 mo liver biopsies (2 samples of 1 cm x 1 mm) will be taken under general anesthesia as follows:

An approximate 2 cm skin incision is made in the ventral abdomen extending from the xiphoid caudally. The abdomen is opened and a hepatic lobe is isolated and partially exteriorized. Biopsies will be obtained using either a true-cut biopsy tool (18 gauge 2-3 sites) or a wedge biopsy (apx 1 cm triangle) will be obtained using sharp dissection and cautery. Biopsy technique will be determined by ease of access and tissue volume requirements. Once hemostasis is confirmed, the abdomen will be closed routinely with absorbable suture.

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>CON</td>
<td>Fasted, blood drawn, oral gavage (2), surgical liver biopsy (2)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>+Cu</td>
<td>Fasted, blood drawn, oral gavage (2), surgical liver biopsy (2)</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
Categories of invasiveness

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

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

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

The rhesus monkey has many similarities in milk profiles and gastrointestinal function with human infants. Additionally, the period of lactation is similar to that of humans and unlike other animal models, no special modifications of infant formula are needed in order to maintain their long-term health. This makes this model ideal for assessing the effects of dietary components on infant health.

Due to previous research in this area, we have determined that 5 animals/group gives acceptable standard deviations as a function of inter-animal variability. Additionally, due to the potentially toxic level of copper these animals will be exposed to, the outcome variables are significantly defined.

Due to the number of infants available for surgical studies and the desire to reduce the number of infants exposed to the elevated copper diet, 5 control animals and 5 copper supplemented animals will be used.

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

Building: CRPRC  
Room: TO BE ASSIGNED

Who will be the surgeon?  
TO BE ASSIGNED BY CRPRC

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>M. mulatta</td>
<td>ketamine</td>
<td>10</td>
<td>IM</td>
<td>As needed by vet</td>
</tr>
<tr>
<td></td>
<td>isofluorathane</td>
<td></td>
<td>inhaled</td>
<td>At 9 and 12 mo (biopsy)</td>
</tr>
<tr>
<td></td>
<td>oxymorphone</td>
<td>0.15</td>
<td>IM TID</td>
<td>At 9 and 12 mo for 3 d(post-operative pain)</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?

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)

Reduced growth
Aspiration pneumonia for oral gastric tube feeding
Pain due to blood draws, liver biopsies
Acute distress due to short-term restraint or individual housing (24 hours) for urine collection
Hematoma due to blood draws

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.

Failure to thrive will result in removal of animal from project
Aspiration pneumonia will be treated by vet staff as needed
Unusual pain or hematoma will be assessed and treated by vet staff as needed

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:

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? 9/20/02

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>Medline/Biosys</td>
<td>1990-2002</td>
<td>Copper toxicity, infants, children</td>
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</table>
What were your findings with respect to alternative methodologies?

Reduction: Due to our previous research in this area, we have determined that 5 animals/group will give us acceptable standard deviation.

Replacement: The rhesus monkey has many similarities in milk profile as well as gastrointestinal function with human infants. Additionally, the period of lactation is similar to that of humans. Unlike other animal models, no special modifications of infant formula are needed in order to maintain their long-term health. This makes this model ideal for assessing the effects of dietary components on human infant health without having to use human infants.

Refinement: This protocol allows us to use the fewest number of animals to determine significance of outcome variables.

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.

NA

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.

<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>M. mulatta</td>
<td>overdose</td>
<td>pentobarbital</td>
<td>60mg /kg</td>
<td>IV</td>
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</tbody>
</table>

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

Returned to the 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>
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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:

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

FinalDispositionofthisprotocol:

___________ Approved

___________ Not Approved

___________ WithdrawnbyInvestigator

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, radionuclides, carcinogens, or toxic chemicals in the animal room.

<table>
<thead>
<tr>
<th>PROTOCOL #________</th>
<th>EXPires:________</th>
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</thead>
</table>

RUA#: 1019  BUA#: ________  CCA#: ________

Identity of Hazard: **Radioactive 67Cu**

Investigator Last Name: [ ]
First Name: [ ]
Department: [ ]
Phone: [ ]
Email: [ ]
Fax: [ ]

**Provide a short description of the agent:**

Radioactive copper isotope with a half-life of 4 days.

**This agent / material is hazardous for:**

- [ ] Humans only
- [ ] Animals only
- [X] Humans and Animals

For which Animal Species?

- [ ] Blood
- [X] Feces/urine
- [ ] Saliva/nasal droplets
- [ ] Does not leave animal

Describe any human health risk associated with this agent:

All radioisotope constitute a health risk. However, this isotope has a very short half life and thus presents a low risk to both the animals as well as handlers.

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.
  - [X] Cage
  - [X] Bedding
  - [ ] Stall
  - [ ] Water Bottle
  - [ ] Animal Carcasses
- [ ] Cages must be autoclaved before cleaning.
- [X] Label cages and remove label after decontamination.
- [ ] Animal carcasses must be labeled and disposed of as follows:
  - [ ] Incineration
  - [ ] Bag and Autoclave
  - [X] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows
    - [ ] Incineration
    - [ ] Bag and Autoclave
    - [X] EH&S will pick-up (2-1493).
- [X] EH&S will pick-up (2-1493).

**Personal Protective Equipment Required:**

- [X] The following personal protective equipment must be worn/used in the room:
  - [X] Lab Coat/Coveralls
  - [X] Disposable Gloves
  - [ ] NIOSH Certified Dust Mask
  - [X] Eye Protection/Face Shield
  - [X] Fitted Respirator
  - [X] Shoe Covers/Booties
  - [ ] Head Cover
  - [ ] Disinfectant footbath
  - [ ] Type: [ ]
  - [ ] Other: [ ]
  - [ ] Describe:

- [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
- [ ] 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: