

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CNPRC**

EH&S USE ONLY

PROTOCOL # 10322**EXPIRES:** _____**Investigator**

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

Contact

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
After hrs. #:	

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

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

Overnight housing location:	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

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)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" 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):	⁶⁷ Cu
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:	Infant formula manufacturer	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	NA

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) 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.

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:

<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 checked="" 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.)

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 from 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.

Group	Procedures / Drugs	Number of Animals	Category
CON	Fasted, blood drawn, oral gavage (2), surgical liver biopsy (2)	5	3
+Cu	Fasted, blood drawn, oral gavage (2), surgical liver biopsy (2)	5	3

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>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.</p>
2	<p>Minor stress or pain of short duration</p> <p>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</p>
3	<p>Moderate to severe distress</p> <p>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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>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.</p>

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.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
M. mulatta	ketamine	10	IM	As needed by vet
	isofluorathane		inhaled	At 9 and 12 mo (biopsy)
	oxymorphone	0.15	IM TID	At 9 and 12 mo for 3 d(post-operative pain)

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 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.

Database Name	Years Covered	Keywords / Search Strategy
Medline/Biosys	1990-2002	Copper toxicity, infants, children

Pub med	1995-2002	Copper toxicity, infants, children
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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 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.

Species	Method	Drug	Dose (mg/kg)	route
M. mulatta	overdose	pentobarbital	60mg /kg	IV

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

Returned to the colony

ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # _____**EXPIRES:** _____RUA#: 1019

BUA#: _____

CCA#: _____

Identity of Hazard: Radioactive 67Cu

Investigator Last Name: _____ Department: _____

First Name: _____ 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 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:

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.
 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: