**CNPRC**

**Last Name:**
**First:**
**Middle:**
**email:**
**Department:**
**Phone:**
**Fax:**

**Species (common names):**

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus monkey</td>
<td>8</td>
<td>Primate Center</td>
</tr>
<tr>
<td>(dams+infants)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project Title:** Preventing intestinal villous atrophy in neonates: A rhesus model

**Overnight housing location:** Primate Center

**Animals will be maintained by:**
[ ] 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.

These studies focus on the use of a novel formulation (“SAFEstart”) proposed for use in human neonates delivered prematurely and at risk for feeding intolerance and intestinal disease. Newborn monkeys will receive SAFEstart or saline for 4 days orally then animals will be euthanized for extensive tissue analyses.

**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.

**Special feeding requirements – see protocol. NO STANDARD DIETS OR SUPPLEMENTS PERMITTED.**

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

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

<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>[ ] Yes [x] No</td>
<td>[ ] Yes [x] No</td>
<td>[ ] Yes [x] No</td>
</tr>
</tbody>
</table>

Agent(s):
Is the project already funded? [X] Yes  [ ] No  
Previously approved? [ ] Yes  [X] No  
Funding Source: Nestle/U Florida  
Previous protocol number (if any):  

What Veterinarian or veterinary clinic will provide care for your animals? (check one)  
[ ] Lab Animal Health Clinic (2-0514)  
[ ] VMTH Large Animal Field Service (2-0292)  
[ ] California Primate Research Center (2-0447)  
[ ] Another Veterinarian  

If you checked “Another Veterinarian”, please provide:  
Veterinarian:  
Address:  

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pctillman@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.  

Human neonates delivered prematurely are generally not fed orally for the first several days of life, rather parenteral nutrition (intravenous fluids) is used to provide the nutritional needs. When a neonate that has not been fed orally for several days begins enteral feedings, feeding intolerance is extremely common, which is, in part, due to intestinal atrophy. A novel preparation has been developed (“SAFEstart”) which is a sterile, isotonic, electrolyte and albumin-containing solution that simulates amniotic fluid and contains essential growth factors present in the amniotic fluid (erythropoietin [Epo], granulocyte-colony stimulating factor [G-CSF], insulin, and L-arginine), and is aimed at preventing such outcomes. The pilot project proposed is designed to assess the outcome after administering this formulation orally for a 4-day duration in newborn monkeys. Solid rationale and convincing studies in humans and animals indicate that oral administration of the components of SAFEstart could benefit preterm neonates since this preparation simulates nature (found in amniotic fluid and colostrum). If the results of this study indicate preservation of villous structure and/or function, this will provide the essential justification to proceed with a Phase I clinical trial in human preterm neonates, for whom SAFEstart has been designed.  

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  
[ ] Special diets; food or water treatment.  
[ ] Induced illness, intoxication, or disease  
[ ] Death as an endpoint (see h below)  
[ ] Multiple survival surgery  
[ ] Trapping, banding or marking wild animals  
[ ] Behavioral modification.  
[ ] 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.)

Four gravid animals and their respective offspring will be included in these studies. They will be sonographically monitored during gestation to confirm normal growth and development of the fetus, with periodic maternal blood samples collected (3-10 ml, femoral vessel; approximately 4-6 times during pregnancy, with all volumes within the acceptable CNPRC limits based on body weight). Animals will be scheduled for a cesarean-section, and the newborns delivered at approximately 150 days gestation. We have delivered newborns at this gestational age many times previously, and these infants can be readily reared in the nursery. Cord blood samples will be collected (~8-10 ml; CBC, chemistry panel, serum and plasma), and an indwelling catheter placed (cephalic or saphenous; placed and maintained by a senior veterinarian), then all infants will be reared in the nursery for the duration of the study. Neonates #1 and #2 will receive SAFEstart (20 ml/kg/day; contains insulin, Epo, G-CSF, L-arginine and albumin, sodium chloride, sodium acetate, potassium chloride; stored in sterile 30 ml vials) using the standard pet feeding bottles, and neonates #3 and #4 will receive normal saline. Infants will be fed every ~2 hrs over each 24-hr period using the standard CNPRC feeding protocol with SAFEstart or saline. All animals will also be administered normal saline intravenously (D10 @ 80 ml/kg/day) for a total intake of 100 ml/kg/day (oral + IV), with the catheters and fluids maintained by the veterinary staff. All animals will be closely monitored for any signs of emesis to ensure the intended ‘dose’ is received, and to avoid any adverse effects. On the morning of day 5, the animals will be administered ketamine, blood samples collected (~5 ml; CBC, chemistry panel, serum and plasma), and the animals euthanized for tissue harvest. Because multiple tissues will be obtained from each animal, two animals per group will be sufficient to address the questions proposed.

SAFEstart or saline + IV fluids

Birth | day 2 | day 3 | day 4 | nx
(day 1) | (day 5)

: 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>Delivery of newborn by cesarean-section, placement of indwelling catheter, oral formulation (SAFEstart) and IV fluid support (newborn), tissue harvest day 5</td>
<td>2 dams + 2 infants=4</td>
<td>3 (dam) 2 (infant)</td>
</tr>
<tr>
<td>2</td>
<td>Delivery of newborn by cesarean-section, placement of indwelling catheter, saline orally and IV (newborn), tissue harvest day 5</td>
<td>2 dams+2 infants=4</td>
<td>3 (dam) 2 (infant)</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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

This is a pilot study to determine the effectiveness of this second generation SAFEstart formulation. Since this is a small exploratory trial, at no time will the outcome be declared as proof of SAFEstart efficacy. The data will be entirely descriptive and evaluated as individual data from each animal. This study will permit direct testing of micro and macro morphometrics of the gastrointestinal tract, and thus provide valuable information relevant to the study hypothesis, and pertinent to extending this approach to human neonates. Monkeys are essential for this study because of similarities when compared to humans, and the need to assess outcomes prior to testing in human infants. Intestinal tissue is needed to directly quantify the effect of this new formulation on intestinal structure. The results of this study will be most meaningful with multiple biopsies collected from multiple anatomic sites at the time of necropsy including stomach, duodenum, jejunum, ileum, and colon. This precludes performing such a study on human neonates where even one biopsy would not be justifiable.

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

<table>
<thead>
<tr>
<th>Building:</th>
<th>Room:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNPRC animal quarters</td>
<td>Surgery suite</td>
</tr>
</tbody>
</table>

Who will be the surgeon?  
CNPRC veterinarians

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>Rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>dams: ~5x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5-8</td>
<td>IM</td>
<td>dams ~3 times</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Isoflurane</td>
<td>to effect</td>
<td>inhal.</td>
<td>Cesarean-section, 1x</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>Post-surgery for dams, 2-3x</td>
</tr>
</tbody>
</table>
Rhesus   | Buprenorphine | 0.01-0.03 | IM | Post-surgery for dams

**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)

We do not anticipate any adverse effects based on prior studies and the short duration of the study. Prior studies by other investigators that focused on improving feeding intolerance of human preterm infants and reducing the morbidities such as with necrotizing enterocolitis (NEC) included administration of an oral preparation of insulin [1, 2002] and L-arginine [et al., 2002], neither of which resulted in adverse effects, with fewer cases of NEC and feeding intolerance observed. Since specific receptors for insulin, Epo, and G-CSF are all expressed on the luminal surface of the neonatal enterocyte, and since these factors are present in human and nonhuman primate amniotic fluid and colostrum, we are confident that there will be no adverse findings. SAFEstart is produced by the All Children’s Hospital Pharmacy Department at the University of South Florida in accordance with strict GMP guidelines. Under all conditions, extreme care will be taken to maintain the sterility and patency of the catheters. However, it is possible that, in some cases, loss of patency will require replacing the catheter. The choice of alternate site will be at the discretion of the senior veterinarian in consultation with the investigators. In addition, antibiotics will be considered, should they be deemed necessary, and provided that the choice of antibiotic does not interfere with the study objective.

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.

See comments above. There are no other adverse effects anticipated or procedures planned that would require administration of analgesics or anesthetics other than those described above. Should any unexpected adverse outcomes arise, they will be addressed immediately by either the appropriate clinical treatments or euthanasia, with decisions made by the investigator and senior veterinary staff working together.

*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?  7/1/03

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

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>1980 to current</td>
<td>Premature infant, animal models, enteral nutrition, feeding, feeding intolerance, amniotic fluid, supplements, villous atrophy</td>
</tr>
<tr>
<td>Reference Update®</td>
<td>Most recent publications</td>
<td>Premature infant, animal models, enteral nutrition, feeding, feeding intolerance, amniotic fluid, supplements, villous atrophy</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are none that would allow us to investigate the questions we propose to address. A primate model is essential to assess the relevance of the proposed feeding protocol for human application.

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.

The studies outlined above are novel and have not been conducted in the manner we propose.

k) Disposition of animals: At what point in the study, if any, will the animals be euthanized?

Infants will be euthanized at day 5 of age. The dams will be returned to the breeding colony two weeks post-cesarean-section.
l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

See comments above.
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/](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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Professor</td>
<td>7/3/03</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CNPRC Director</th>
<th>Date</th>
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</table>

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.

<table>
<thead>
<tr>
<th>Campus Veterinarian</th>
<th>Date</th>
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<tbody>
<tr>
<td></td>
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</table>