

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

EH&amp;S USE ONLY

**PROTOCOL: 10223  
EXPIRES:**

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:
<i>Macaca mulatta</i>	60	CRPRC, field colony and nursery raised

Project Title	Effect of feeding cruciferous vegetables on <i>Helicobacter pylori</i> infection in Rhesus macaques.		
Overnight housing location::	CRPRC	Day use only :	
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.

The extent of <i>Helicobacter pylori</i> infection will be assessed by taking stomach biopsy samples before, during and after supplementing the animal's diet with cruciferous vegetables for a period of weeks. Both naturally and experimentally infected animals will be used.
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**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.

For the study period, the fresh produce the animals usually receive will be replaced with fresh broccoli or other fresh cruciferous vegetables provided by the investigator. No cruciferous vegetables (broccoli, cauliflower, kale, cabbage or brussel sprouts) can be fed to the animals one month prior to and throughout the feeding study other than the broccoli provided by the investigator.
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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 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 type="checkbox"/> Necropsy	

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

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	<i>Helicobacter pylori</i>
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
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:

UC Davis Clinical Nutrition  
Research Unit

Previously approved?

 Yes  No

Is the project already funded?

 Yes  No

Previous protocol number (if any):

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

If you checked "Another Veterinarian", please provide:

Veterinarian:

Address:

Day phone:

Email:

Emergency phone:

*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.

Infection with *Helicobacter pylori* causes a histological gastritis that in some individuals is associated with the development of peptic ulcer disease or gastric malignancy. Several studies have demonstrated an anticancer effect due to isothiocyanate compounds naturally present in cruciferous vegetables such as broccoli, cauliflower, kale and brussel sprouts. One of these compounds, sulforaphane, was recently demonstrated to have antimicrobial properties against *Helicobacter pylori* (*H. pylori*). Some strains of broccoli contain more concentrated amounts of sulforaphane. While this effect has been demonstrated *in vitro*, no antimicrobial effect has been shown *in vivo*. Rhesus monkeys are naturally infected with *H. pylori* that is very similar to strains that infect humans, and this animal model provides a unique opportunity to study the effect of dietary compounds on bacterial load and the possibility of prevention of infection with dietary measures. The rhesus macaque model system facilitates research studies that cannot be performed readily in human patients.

**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 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.)

**Specific Aim 1: Determine the effect of broccoli feeding on *H. pylori* bacterial load in naturally infected rhesus macaques.**

**Animals:** All experimental animals will be colony-bred rhesus monkeys of either sex, negative for antibody to SIV, STLVs and SRV (a simian Type D immunosuppressive retrovirus). 10 adult rhesus macaques (4-10 years of age) that are naturally infected with *H. pylori* will be selected by screening banked or fresh serum of colony animals. As the infection rate by 4 years of age is nearly 100%, we do not expect to screen more than 15 animals to find 10 seropositive candidates for the study.

**Design:** In order to determine a baseline *H. pylori* infection level for each animal, endoscopic gastric biopsy will be performed two to three times at two week intervals prior to initiation of the feeding study. If a similar number of colony forming units per gram (cfu/g) of tissue (within 20% of each other) are obtained for the first two timepoints, a third will not be required. The feeding study will begin on the Monday following the determination of culture results. During the pre-feeding study period no cruciferous vegetables (broccoli, cauliflower, kale or brussel sprouts) are to be offered to the animals, however other fresh fruits and vegetables, part of their normal diet, will be permitted.

Monkeys will be pair-housed indoors for the four week feeding study. Broccoli provided by the investigator will be offered five days a week (Monday through Friday). The broccoli will replace the fruits and vegetables normally offered to the animals three times a week (Monday, Wednesday and Friday). The volume of fresh broccoli offered will be about ¼ cup, similar to the amount of fresh vegetables and fruit normally offered. In order to verify that each individual is consuming its share of broccoli the animals will be separated when this food is offered. The animals typically eat the fresh food in minutes and can be reunited quickly. Prior to putting the animals back together, visual inspection of the animal's cage and the surrounding area will be made by the animal care staff to determine whether the broccoli has been consumed. A worksheet will be provided by the investigators for the animal care staff to record the results of visual inspection.

Broccoli is eaten readily by rhesus macaques at the CRPRC and we do not anticipate any problem in getting the animals to eat the proffered vegetable. If the monkeys tire of broccoli due to the frequency of its being offered, we will consider decreasing the frequency to 3 days a week.

To determine the effect of broccoli feeding on *H. pylori* colonization, each animal will be anesthetized for endoscopic gastric biopsy every two weeks (see schedule below).

Gastric biopsy schedule with 3 pre-feeding biopsies:

-4 wk   -2 wk   0 wk   2 wk   4 wk   6 wk

Gastric biopsy schedule with 2 pre-feeding biopsies:

-2 wk   0 wk   2 wk   4 wk   6 wk

If no effect is observed at the 4 wk timepoint after the start of the feeding study, the 6 wk timepoint will be dropped. If a decrease in bacterial load is observed, endoscopic gastric biopsy will be continued at monthly intervals for up to one year.

**Methods:**

(1) **Serum collection.** Banked serum will be used when possible for screening for *H. pylori* antibodies. If it is more efficient to collect a fresh sample from candidate animals for the study, 5 ml of blood will be obtained one time by saphenous or femoral venipuncture. Sera will be aliquoted and frozen at -70°C. No additional blood samples are anticipated.

(2) **Endoscopic gastric biopsy.** Animals will be fasted overnight and given ketamine anesthesia (10 mg/kg) im. Eight to twelve gastric biopsies will be collected (antrum and body) at each endoscopy. The endoscope will be thoroughly disinfected after each procedure.

(3) **Urine collection.** Urine will be collected at each endoscopic gastric biopsy by needle tap into the bladder for quantitation of sulfuraphane degradation products by HPLC.

(4) **Broccoli Preparation.** The Brigadier strain of broccoli (commercially available seed stock) will be grown by

the laboratory of Carlos Quiros, Ph.D. in the Vegetable Crops Department of the University of California, Davis. This strain has a glucoraphanin (sulforaphane's pre-compound) content of 21.7  $\mu\text{mol/g}$  dry weight.

**(5) Disposition of animals.** Animals naturally infected with strains of *H. pylori* enzootic at the CRPRC will be returned to the colony without antibiotic treatment.

### **Specific Aim 2: Determine the preventative effect of broccoli feeding on *H. pylori* reinfection.**

**Animals:** All experimental animals will be colony-bred rhesus monkeys of either sex, negative for antibody to SIV, STLVs and SRV (a simian Type D immunosuppressive retrovirus). 20 adult rhesus macaques (4-10 years of age) will be selected for this Aim. Monkeys will be screened for *H. pylori* infection by serology. It is expected that all candidate animals will be seropositive for *H. pylori*. In order to have a consistent starting point for this aim, only seropositive animals will be selected.

**Design:** There will be ten animals in the test group and ten animals in the control group. All twenty animals will be treated to eradicate *H. pylori* infection. Eradication of infection will be verified by endoscopic gastric biopsy one time, approximately 4 weeks after completion of eradication therapy. This method of eradication is almost 100% effective in previous studies. No cruciferous vegetables other than the broccoli provided by the investigators will be given to the study animals during the study period, beginning with treatment and ending with the last biopsy. Broccoli feeding will begin for the test group two weeks prior to inoculation with *H. pylori* strain J166. As with the feeding study described in Aim1, broccoli will be offered five times/week to the test animals in place of their normal fresh fruit and vegetables. The control animals will receive a normal diet, including fresh fruit and vegetables (except broccoli and other cruciferous vegetables) three days a week. Both the test and control animals will be inoculated with either  $10^5$  (n=5 test animals, n=5 control animals) or  $10^6$  (n=5 test animals, n=5 control animals) cfu of *H. pylori* J166. Endoscopic gastric biopsy will be performed every two weeks for twelve weeks post-inoculation. At this time infection should be stabilized and an effect of preventative feeding of broccoli would be apparent.

**Methods:** In addition to the methods listed for Specific Aim 1, the following methods apply to monkeys included in Specific Aim 2.

**(1) Treatment to eradicate *H. pylori* infection.** Naturally infected monkeys will be treated by gavage with antibiotics to eradicate *H. pylori* infection prior to the feeding period and inoculation with a human strain of *H. pylori*, J166. Omeprazole, 0.4 mg/kg; clarithromycin, 11 mg/kg; bismuth, 20 mg/kg; amoxicillin, 14 mg/kg will be given twice daily for 10 days to clear *H. pylori*.

**(2) Inoculation of cultivated *H. pylori*.** Inoculation of  $10^5$ - $10^6$  cultivated *H. pylori* will be performed with human-derived strain J166. Inoculation will be by the orogastric route after an overnight fast. Anesthesia will be required for inoculation. Inoculation volume will be 2 to 3 mls.

**(3) Disposition of animals.** After completion of the proposed experiments, all experimentally infected animals will be treated with antibiotics (omeprazole, 0.4 mg/kg; clarithromycin, 11 mg/kg; bismuth, 20 mg/kg; amoxicillin, 14 mg/kg) twice daily for 10 days to clear their *H. pylori* infection. Eradication will be verified by endoscopic gastric biopsy approximately 4 weeks after the completion of therapy and the animals returned to the colony.

### **Specific Aim 3: Determine the antimicrobial effect of sulforaphane in naturally infected rhesus macaques.**

**Purpose:** Broccoli contains several types of isothiocyanate compounds. Some of these compounds, such as the indole glucosinolates do not appear to have antimicrobial properties and may even degrade into compounds that are tumorigenic. Broccoli sprouts have very high quantities of sulforaphane and a low content of indole glucosinolates. Due to the anticipated difficulty in getting the monkeys to consume a set amount of broccoli sprouts, we propose to administer the sulforaphane compound alone.

**Animals:** All experimental animals will be colony-bred rhesus monkeys of either sex, negative for antibody to SIV, STLVs and SRV (a simian Type D immunosuppressive retrovirus). 18 adult rhesus macaques (4-10 years of age) naturally infected with *H. pylori* will be selected. As described for Aim 1, animals will be selected based on positive *H. pylori* infection status as determined by serology performed on a banked or fresh serum sample. We anticipate screening approximately 25 animals to find 18 seropositives.

**Design:** There will be six monkeys in each dosing group for three doses of sulforaphane. Although the biologically relevant amount of sulforaphane is not known, a 'dose' can be extrapolated from considering the normal content found in these vegetables at different stages of growth and previous studies performed in rodents. In addition, the minimum inhibitory concentration (MIC) of sulforaphane against *H. pylori* against 90% of 48 *H. pylori* strains tested has recently been determined to be 4  $\mu\text{g/ml}$ . This study also involved administration of

sulforaphane to 9-12 week old mice at a rate of 7.5  $\mu\text{mol/day}$  by adding 2.5 mmol (442.5 mg) sulforaphane/kg of food.

The baseline *H. pylori* bacterial load will be established for each animal by endoscopic gastric biopsy every two weeks, two to three times as described for Specific Aim 1. Sulforaphane administration will begin on the Monday following the establishment of bacterial load. No cruciferous vegetables will be given to the animals during the study period. The three doses of sulforaphane administered to the monkeys will be 50, 75 or 100  $\mu\text{mol/day}$ . Six animals will make up each dosing group and will receive sulforaphane 5 days a week for four weeks. Endoscopic gastric biopsy will be performed every two weeks as described for Specific Aim 1.

**Methods:** In addition to the methods described above for Specific Aim 1, the following methods apply to monkeys included in Specific Aim 3.

**(1) Administration of sulforaphane.** 50, 75 or 100  $\mu\text{mol}$  sulforaphane compound will be diluted in a small volume (2-5 ml) of Tang or sugar water and administered to the monkeys daily. The compound will be administered by gavage to anesthetized animals, unless the animals can be trained to take the small volume offered by a feeding syringe.

**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	Broccoli feeding, gastric biopsy	15	2
2	Treatment to eradicate <i>H. pylori</i> infection, broccoli feeding, gastric biopsy and experimental <i>H. pylori</i> infection	20	2
3	Isothiocyanate compound administration, gastric biopsy	25	2

## 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?

Choice of rhesus is based on the fact that this species is the only nonhuman animal that is naturally infected with *H. pylori*, making it particularly relevant. The group of ten animals for Aim 1 is based on the animal to animal variation in the initial *H. pylori* bacterial load. Most group comparisons are based on sample sizes of 4-5, which is the minimum to make meaningful statistical comparisons. This larger group size was chosen because we expect some variability in the pre-feeding bacterial load and we want to look for differences in bacterial load after feeding broccoli. This requires larger samples. Aim 2 will include ten animals in the test group and ten control animals. Our previous studies demonstrate we can reinfect a treated monkey with *H. pylori* by inoculating with 10(5) c.f.u. of *H. pylori*. Again we will see variability in the bacterial load post-infection. A larger group size is necessary for both the test and control groups to minimize the effect of this variability. Aim 3 will use groups of 6 animals for each dose of sulfuraphane. Considering the variability in natural infection we expect to see, a group size of 6 is the absolute minimum to derive useful information regarding the effect of this compound on *H. pylori* bacterial load.

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?
<i>M. mulatta</i>	Ketamine	2.5-10	im	Prior to endoscopy

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)

Mild gastrointestinal upset may occur related to the frequent feeding of cruciferous vegetables, but should not cause the animal distress.

Very rarely, gastric endoscopy can result in esophageal or gastric perforation, which requires surgical repair. Risk of perforation or bleeding from endoscopy is thought to be about 1/5000. Occasionally there can be tracheal edema from endotracheal intubation, which may requires a dose of dexamethasone and sometimes a brief (1-2 hour) period of oxygen delivery.

No significant clinical signs have been associated with experimental inoculation with *H. pylori* in rhesus macaques.

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.

We do not anticipate the need for analgesics or other therapies related to the proposed experiments. However, it is up to the discretion of the veterinary staff of the CRPRC to treat any animal on the study that exhibits clinical signs of illness.

*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: [mwwood@ucdavis.edu](mailto:mwwood@ucdavis.edu))*

What was the date on which you conducted this search?

7/3/02

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
PubMed/Medline	1965-2002	<i>Helicobacter</i> , primate, sulforaphane, isothiocyanate
Primate Information Center, Univ. of	1940-2002	<i>Helicobacter</i>

Washington <a href="http://primatelit.library.wisc.edu/">http://primatelit.library.wisc.edu/</a>		

What were your findings with respect to alternative methodologies?

There have been no studies of the effect of cruciferous vegetable feeding on *H. pylori* infection.

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.

N/A

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

N/A

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
<i>M. mulatta</i>	Lethal injection	Pentobarbital	60 mg/kg	IV

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

Return to the colony.



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.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

<b>** Conditions necessary for Committee Approval:</b>
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> 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.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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**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#: \_\_\_\_\_

BUA#: 0524

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

Identity of Hazard: *Helicobacter pylori*

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

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

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

**Provide a short description of the agent:***Helicobacter pylori* naturally infects the stomach of some rhesus macaques and humans.

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

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

**Describe any human health risk associated with this agent:**

*H. pylori* is a human pathogen that is associated with ulcers and gastric cancer. However, infection in humans is common (50% of persons 50 yrs of age or older are infected) and 85-90% of infected persons have no clinical disease. The mechanism of spread is not known, but may involve ingestion of contaminated feces or vomitus. Aerosol spread from a vomiting animal is possible but unlikely unless one is very close (1-2 ft). *H. pylori* has no known adverse effects on rhesus monkeys, and they are universally infected in the colony by age 3-4. Risk of human infection with *Helicobacter pylori* from the monkeys is very low. All the necessary precautions taken to prevent human infection with B virus should protect people working with *Helicobacter pylori* infected monkeys.

**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: N/A

Date: March 13, 2003

To: Animal Care and Use Administrative Advisory Committee  
c/o Office of the Campus Veterinarian  
and the California National Primate Research Center

From:

RE: Amendment to Protocol #10223

Proposed Changes:

A issue has arisen that requires an amendment to our proposed methods for determining the effect of feeding cruciferous vegetables on *Helicobacter pylori* infection in rhesus macaques. We had proposed to feed the monkeys broccoli with a defined glucosinolate content grown by our collaborator here at Davis. The warm winter we experienced limited the growth of the broccoli and we do not have enough to complete the study. For this reason, we would like to offer 3-5 day old broccoli sprouts instead of broccoli. The glucosinolate content will be similar to that proposed, approximately 100  $\mu\text{mol}/\text{animal}/\text{day}$ .

As sprouts are not palatable to the monkeys we need to administer a slurry of broccoli sprouts blended in water by a feeding tube. The volume of the slurry will be limited to that necessary to deliver the desired glucosinolate content in enough water to allow it to flow through the feeding tube easily (approximately 20 ml). The sprout slurry will be administered one of two ways:

(1) the animals will be hand caught and restrained while the sprout slurry is administered according to the previously proposed schedule of five days a week (Monday through Friday) for a total of four weeks (20 days of receiving broccoli sprouts).

(2) a low dose of ketamine anesthesia will be given to chemically restrain the animals. If ketamine anesthesia is given the animals will only be given the sprout slurry every other day. If anesthesia is used, we would like to extend the study period an additional week (to 5 weeks) to provide more days of feeding with the every other day protocol. We would deliver the sprout slurry Monday, Wednesday and Friday of every week for a total of 15 days of broccoli sprouts.

Justification:

As described above the broccoli we had proposed to give to the animals is not available. Another method to deliver a similar glucosinolate amount is necessary.

Potential adverse effects:

We do not foresee any adverse effects to the proposed changes. The use of ketamine anesthesia could depress the appetite of the animals. For this reason we propose to use anesthesia every other day. The decision whether to use anesthesia for the study will depend on the availability of animals infected with *H. pylori* that are sufficiently small to safely hand catch. Some of the animals that we have screened are too large to hand catch safely.

Date: Wed, 26 Mar 2003 16:12:37 -0800  
To:  
From:  
Subject: Answers to amendment 10223 questions

The answers to the questions are attached. Please let me know if there are any other questions. I will be out tomorrow morning, but will address any additional questions asap.

Amendment to Protocol 10223

Regarding the proposed change requiring the restraint/anesthesia and tubing of the animals with a slurry of broccoli sprouts-

1. Which group are you proposing to treat this way? Which "aim" as specified in the protocol does it serve?  
We are proposing to treat the animals of Aim 1 with the broccoli sprout slurry.

2. On page 4, section c of the protocol, you describe the difference in content of the broccoli vs. the broccoli sprouts and state that the content of the sulforaphane and glucosinolate are different between the two different forms of the vegetable. Now it appears that you are proposing to substitute the sprouts for the mature vegetable, when you have already stated that they are different. How is this justified within the design of the study?  
Both mature broccoli and broccoli sprouts contain glucosinolate compounds, however, broccoli sprouts contain a higher amount per unit weight. We are proposing to give the animals the same amount of the glucoraphanin (glucosinolate form of sulforaphane) compound (i.e. 100  $\mu$ mol/animal/feeding day). Broccoli sprouts are perhaps a better source of glucosinolates than mature broccoli due to this high glucosinolate content and the lack of some of the indole glucosinolates that occur in mature broccoli. We had proposed to start with the mature vegetable as it would have been more straightforward.

3. Why can't you use another source of broccoli, rather than the sprouts, removing the need to restrain/anesthetize and tube the animals so frequently?  
As explained in the amendment, we do not have a consistent source of mature broccoli that contains a high amount of glucoraphanin. We cannot use broccoli off the supermarket shelves as it varies widely in glucoraphanin content. The warm winter prevented growth of enough broccoli for the four week study. Broccoli sprouts are readily available as they only take 5 days to grow. The monkeys did not find them palatable, however, which requires that they be presented by tube feeding.

Tube feeding the animals will also ensure that each animals receives the full amount of broccoli sprouts – an improvement in the study design when considering data analysis.

In addition, we found it more difficult than anticipated to identify even 5 animals that had a sufficient level of *H. pylori* infection to qualify for the study. Aim 1 was written to use 10 monkeys. We have only identified 5 and will likely only use 5 for this part of the study. Were infected animals more readily available, we would select 5 animals that could be hand caught and avoid the need for anesthesia.

4. Is there any risk that ketamine-anesthetized macaques could regurgitate and aspirate the broccoli slurry?

There is a risk of regurgitation of the slurry in anesthetized animals. We will minimize the volume administered and monitor the animals carefully to prevent this from happening. We will use a light dose of ketamine to make the animals safe to handle, but limit the recovery time.