

PROTOCOL FOR ANIMAL USE AND CARE

*Handwritten forms are not accepted*

**CRPRC**

EH&S USE ONLY PROTOCOL # <u>9713</u> EXPIRES: _____
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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:
Cynomolgus macaque	48 (total for 3 years)	CRPRC colony

Project Title: Antiprogestins in Cynomolgus Endometriosis

Overnight housing location:  CRPRC  Day use only : \_\_\_\_\_

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.

This project will induce endometriosis (a disease of the female reproductive tract) by surgical introduction of endometrial fragments into the peritoneal cavity of female cynomolgus monkeys. Parameters to determine outcome will include menstrual cyclicity, serum and urinary hormones (estrogen, progesterone, cortisol, follicle stimulating hormone [FSH]), endometrial and vaginal tissue sampling, changes in bone function assessed by serum biomarkers and bone density measurements (dual energy X-ray absorptiometry [DEXA]), and changes in liver function assessed by serum markers.

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.

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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 type="checkbox"/> Call Investigator               |
| <input checked="" type="checkbox"/> Clinician to treat | <input type="checkbox"/> Save for Investigator        | <input checked="" type="checkbox"/> OK to use pesticides |
| <input type="checkbox"/> Terminate                     | <input 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 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:	NIH	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

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

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

If you checked "Another Veterinarian", please provide:

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

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

This study will test the hypothesis that a new class of antiprogesterins, namely selective progesterone receptor modulators (SPRMs), will ameliorate experimentally induced endometriosis in the macaque, a well-established reproductive model for humans. The test agent (CDB-4124), a new SPRM, will be compared to two hormone modulators (gonadotropin releasing hormone agonist [GnRHa] and RU 486) in effectively treating endometriosis with reduced side effects. We will use surgical, histological, and endocrine techniques, to evaluate the safety and efficacy of CDB-4124 compared to RU486 and GnRH. Bone and liver function will also be monitored to ensure the safety of the test agent on these systems.

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 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 checked="" type="checkbox"/> Euthanasia                     |
| <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.)

Forty-eight females will be monitored for normal menstrual cycles during a 12-week observation period by visual observation of menstrual bleeding in each animal's respective cage and analysis of urinary hormones. Daily urine samples (~3 cc per timepoint) will be obtained from pans placed below each animal's respective cage. All animals with normal cycle characteristics will then undergo an initial surgical procedure (laparotomy) to inspect for spontaneous abdominal adhesions. We anticipate that 36 to 42 of these initial 48 animals will be acceptable for assignment to the study based on normal cycle hormones and lack of adhesions. Those selected for the study will be randomly placed into six groups of 6-7 animals per group and will undergo the procedures described below. Those unacceptable for the study will be returned to the colony.

During the initial surgery for adhesion inspection, endometriotic lesions will be induced by placing endometrium in five different locations within the peritoneal cavity of all study animals. At the same time uterine biopsies (<5 mm), vaginal smears, peritoneal lavages, and serum for analysis of bone and liver biomarkers will be obtained from these animals. All of these samples will be analyzed as baseline measurements. All laparotomies will be performed while the animal is under anesthesia as detailed in section g.

Four weeks later, study animals will undergo a second laparotomy to inspect endometrial lesions in the peritoneum to establish the baseline level of endometriosis. Treatment will be initiated on the day post-surgery according to the treatment groups summarized below. All treatments will be administered for 36 weeks. The test compound (CDB-4124) and RU 486 will be administered orally (p.o.) via nasogastric intubation on a daily basis. GnRH agonist will be injected (i.m., upper thigh or arm) on a weekly basis. The vehicle will be administered orally (p.o.) via nasogastric intubation on a daily basis and by i.m. injection on a weekly basis.

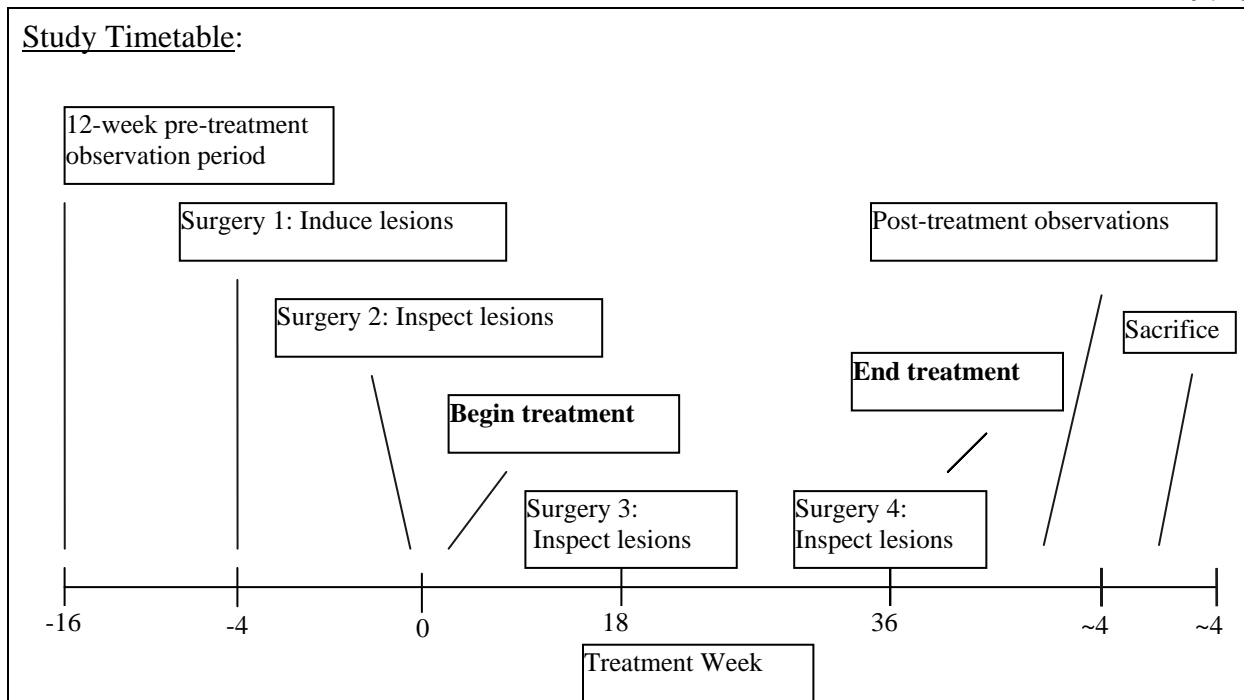
A third laparotomy will be performed after 18 weeks of treatment to inspect endometrial lesions and take a peritoneal lavage sample. A fourth laparotomy will be performed at the end of treatment at 36 weeks to inspect endometrial lesions and obtain a peritoneal lavage sample. At the end of treatment, uterine and vaginal smear samples will also be taken while the animal is lightly anesthetized (ketamine).

Following the 36-week treatment period, there will be a 6-week post-treatment period involving return-to cycling observations. These observations will include continual monitoring of menstrual bleeding and collection of daily urine samples as described for the 12-week pre-treatment observation period.

Serum samples (~5 cc whole blood) for markers of bone formation and bone resorption, cytokines, and liver enzymes will be taken once during week 24 of treatment and once during the 6-week post-treatment period. Blood will be drawn from the cephalic vein using arm-pull technique with a 6cc syringe and 22-gauge needle. Lumbar spine bone density (DEXA) scans and daily urinary samples for hormone analyses will also be obtained during week 24 of treatment and during the 6-week post-treatment period.

All treated and control animals (n=~36-42) will be euthanized at the end of the post-treatment period to perform a full histopathological examination with special emphasis on the reproductive tract. The remaining animals not assigned to the study (n=~6) will be returned to the colony.

There will be six treatment groups (6-7 animals each), as summarized in the table below. There will be three control groups; the negative control group will receive vehicle and the positive control groups will be treated with RU 486 or GnRH, both of which have been used previously to alleviate endometriosis. There will be three treatment groups that will receive the test agent, CDB-4124 - high dose (5 mg/kg/day), mid-dose (1 mg/kg/day), and low dose (0.2 mg/kg/day).



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
Control Vehicle, p.o. daily and i.m. weekly	Surgery to produce endometrial lesions, p.o. and i.m. administration of vehicle, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3
GnRH Lupron, i.m., weekly	Surgery to produce endometrial lesions, i.m. injection with Lupron, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3
RU486 5.0 mg/kg/day, p.o., daily	Surgery to produce endometrial lesions, p.o. administration of RU486, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3
CDB-4124, high dose 5.0 mg/kg/day, p.o., daily	Surgery to produce endometrial lesions, p.o. administration of CDB-4124, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3
CDB-4124, mid-dose 1.0 mg/kg/day, p.o., daily	Surgery to produce endometrial lesions, p.o. administration of CDB-4124, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3
CDB-4124, low dose 0.2 mg/kg/day, p.o., daily	Surgery to produce endometrial lesions, p.o. administration of CDB-4124, blood and urine samples, DEXA, vaginal smear, follow-up laparotomies for uterine biopsies and peritoneal lavages	6-7 total	3

## Categories of invasiveness

Category	Description
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 cynomolgus monkey is a well-established reproductive and developmental model for humans due to qualitative and quantitative similarities in menstrual cycle characteristics, hormonal profiles, and reproductive anatomy. A sufficient number of animals in both control and treated groups (i.e., 6-7) is required to account for variability in normal reproductive and endocrine characteristics. Three doses of the test agent are required to establish a dose response.

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

Building:	CRPRC	Room:	1316
Who will be the surgeon?	CRPRC Veterinary staff		

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?
Cynomolgus Monkeys	Ketamine HCl	10	IM	1x/day/laparotomy
	Atropine	0.04	IM	1x/day/laparotomy
	Isoflurane	To effect	Inhalation	1x/day/laparotomy
	Oxymorphone	0.15	IM	3x/day for 3 days post-laparotomy

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?

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## 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)

Post-hysterotomy discomfort may occur. Based on prior studies at the CRPRC, treated and vehicle-control animals may experience mild transient weight loss and/or poor appetite during the treatment period. Daily monitoring of all animals will ensure maintenance of health and animal well being. Additionally, fruit supplementation will be employed, as needed, to keep the animals well nourished during the treatment period.
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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.

Post-hysterotomy discomfort will be alleviated with oxymorphone.
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*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.*

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## 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?

August, 2001
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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 HealthStar	1980 – 2001	Nonhuman primates, macaques, endometriosis, endometrial lesions, uterus, antiprogestins, SPRM, GnRH agonist, cyclicity, contraceptive agents
Primate Literature Database (University of Washington)	1980 - 2001	Nonhuman primates, macaques, endometriosis, endometrial lesions, uterus, antiprogestins, SPRM, GnRH agonist, cyclicity, contraceptive agents

What were your findings with respect to alternative methodologies?

There are numerous published studies in various animal models on hormonal control of the normal menstrual cycle and descriptions of endometriosis. However, to our knowledge, there are no published articles on treatment of endometriosis using SPRMs in nonhuman primates. The test compound in this study, CDB-4124, is a newly developed and proprietary SPRM which has only been evaluated in a pilot study by this group to determine appropriate dose groups for current study. There are no other published studies on this compound.
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Has this study been previously conducted?

<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
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If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

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

All treated and control animals (n=-36-42) will be sacrificed at the end of the study.

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
Cynomolgus Monkeys	Overdose	Pentobarbital	60 mg/kg	i.v.

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

Animals evaluated at the beginning of the study, but not assigned (n=-6), will be 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.

Last Name	First Name	Middle Name	UC ID Number or SSN	Email Address

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/> 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/>.

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>

Committee Use Only Below

** Conditions necessary for Committee Approval:         
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>