

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

EH&amp;S USE ONLY

**PROTOCOL #10102  
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:
Cynomolgus Macaque (males)	5	CRPRC/PTF Colony

Project Title	DVS-80; Antispermatogenic Activity of CDB-4022C in Male Cynomolgus Monkeys		
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.

Five male monkeys will receive a single oral dose of CDB-4022C in 10%ethanol/90% sesame oil at 12.5 mg/kg BW on day 0. Blood and semen will be collected once per week, three weeks before treatment, with blood collected once per week and semen collected once per two weeks for 20 weeks thereafter. Both testes and epididymides will be removed surgically at weeks 10 and 20 respectively. Testes and epididymides will be examined for evidence of reduced fertility using histology.

**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.

No special husbandry requirements will be required.

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 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 checked="" type="checkbox"/> Yes <input 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) 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.

The purpose of this study is to evaluate the Indenopyridine, CDB-4022C for its antispermatogenic effects in the non-human primate. The significance of this study is that this drug may represent a potential contraceptive for human males.

Previous studies of CDB-4022 in adult male rats given a single oral threshold dose at 2.5 mg/kg BW, induced irreversible infertility ( et al., 2001a). The irreversible nature of CDB-4022 induced infertility is presumably due to its adverse actions on the Sertoli cell ( et al., 2001b). However, in a pilot study in non-human primates, CDB-4022A at 2.5 mg/kg BW did not have an adverse effect on the fertility status of adult male rhesus monkeys as assessed by semen analysis and morphological evaluation of the testes. Any potential antispermatogenic effect of CDB-4022A in monkeys may have been missed due to the low dose used. Therefore, this study will be undertaken at a higher dose in order to determine; whether CDB-4022C induces infertility in adult male cynomolgus monkeys; and whether this antispermatogenic effect, if present, is reversible in monkeys. The dose that induces maximal testicular damage in adult male rats (12.5 mg/kg, et al, 2001a) will be used in this study to maximize the possibility of observing an effect in adult male monkeys. Antispermatogenic effects of CDB-4022C will be assessed in the male cynomolgus monkey by examination of semen samples, measurement of circulating levels of inhibin B, testosterone, mFSH and mLH, and histological evaluation of the testes and epididymides. These parameters will be compared in all five animals before and after treatment with the compound in order to determine the effects from treatment.

**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 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.)

Five adult male cynomolgus monkeys will be placed into a single treatment group. Three weeks prior to treatment, all animals will have semen collected once per week for three weeks by electro-ejaculation. All electro-ejaculations will be done while the animals are un-anesthetized and restrained in a primate chair to which they have been previously trained. Additional semen collections, once every two weeks, will be obtained for twenty weeks following treatment (13 semen collections in all).

On study day 0, all five animals will receive a single dose of CDB-4022C at 12.5 mg/kg body weight orally by naso-gastric intubation, while animals are briefly hand-restrained. The drug concentration will be 12.5 mg/ml in 10% ethanol/90% sesame oil for vehicle.

Blood samples (5 cc/animal/date) will be collected from cephalic, saphenous or femoral vein of animals while briefly cage-restrained once per week during three weeks prior to treatment then continuing through week 20 of the study (23 blood collections).

Surgical removal of the left and right testes and epididymides will be performed at weeks 10 and 20 of the study respectively, while animals are under general anesthesia. Ketamine (10 mg/kg) and atropine (0.05 mg/kg) as pre-medication and maintenance anesthesia with isoflurane will be used for both surgeries. Post-operative pain management after first surgery will consist of Ketaprofin (2.0 mg/kg BW, IM) once a day for two to three days. After the second surgery, Buprenorphine (0.01-0.03 mg/kg) twice per day for 2-3 days following surgery will be used for analgesia. Opioids have known effects on the hypothalamic-pituitary-gonadal axis. Therefore, in order to avoid any interference from the analgesia, ketaprofin has been selected to be used following first surgery. All of these procedures will be done under the guidelines of the Institutional Animal Care and Use Committee. Blood samples collected on weeks 10 and 20 will be obtained before surgery.

At the conclusion of the study on week 20, all animals will be returned the PTF colony at the CRPRC.

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	12.5 mg/kg BW of CDB-4022C (Indenopyridine) at 12.5 mg/mL in 10% ethanol/90% sesame oil by NGT	5	3

## 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?

Cynomolgus monkeys are the most appropriate animal for this study and future contraception studies because they are reproductively similar to humans for which contraceptive drugs are ultimately being developed.

Since these animals will have pre-treatment and post-treatment blood and semen collections obtained, their circulating hormone levels, as well as sperm morphology and motility parameters can be compared in each animal to determine the effect of treatment. Therefore, there is no need for additional animals to be used as controls. Five animals have been selected as a conservative number where an effect of treatment should be able to be assessed, accounting for expected variations in hormone and sperm parameters. A one-way ANOVA will be used on before and after treatment indices for hormones and semen parameters.

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

Building:

CRPRC

Room:

Surgical Suite

Who will be the surgeon?

CRPRC Vet. 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?
M. fascicularis	Ketamine	10	IM	1 time per surgery
	Atropine	0.05	IM	1 time per surgery
	Isoflurane	To effect	Inhal.	1 time per surgery
	Ketoprofin	2.0	IM	1X/day for 2-3 days following first surgery
	Buprenorphine	0.01-0.03	IM	2X/day for 2-3 days following second surgery

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)

Daily monitoring of all animals will be done to ensure maintenance of animals' health. All animals undergoing testicular surgery may experience some moderate but transient discomfort. We do not anticipate any adverse effects from blood, semen collections or naso-gastric intubation treatments, or test article. Fertility in these animals may be compromised from this compound, but previous studies did not report any other adverse effects with respect to health and well-being.

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.

Any post surgical discomfort will be alleviated with ketoprofen or buprenorphine.

*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.*

Death is not an endpoint in this study.  
Euthanasia will be done by clinician's advice.

**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?

4/29/02

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
Current Contents	1993-present	Contraceptive, Indenopyridine, Spermatogenesis
Biosis	1993-present	Contraceptive, Spermatogenesis, Testes, Sperm

What were your findings with respect to alternative methodologies?

No alternative methodologies were found.

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.

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

Euthanasia will be done on clinician's advice.

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. Cynomolgus	as per CRPRC guidelines	Pentobarbital	60 mg/kg	IV

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

All animals will be returned to the PTF colony at the CRPRC at the completion of the study.

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.

Principal Investigator	Rank / Title	Date
CRPRC Director	Date	

**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.

Campus Veterinarian	Date
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