PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

CNPRC

PROTOCOL:

CNPRC

EXPIRES:

EH&S USE ONLY

Investigator

Last Name:

First:

Middle:

e-mail:

Department:

Phone / Fax:

After hrs. #:

Contact

Last Name:

First:

Middle:

e-mail:

Department:

Phone / Fax:

After hrs. #:

Species (common names):

Cynomolgus macaque

Number:

35

Source:

CNPRC

Project Title

In vivo effect of bromodichloromethane (BDCM) on primate placental structure and function

Overnight housing location:

CNPRC

Day use only:

CNPRC

Animals will be maintained by:

[X] 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.

Animals who have been bred for pregnancy will have daily urines (from cage pans) as well as blood collected before and after treatment regimen. Each animal who passes the breeding criteria will receive daily oral doses of bromodichloromethane (BDCM) on gestational days (GD) 12 through 18. A dose finding study will be conducted to determine the target dose. Hysterotomy will be conducted on GD 28 and implantation site tissues and the embryos will be collected. All animals will be returned to the CNRPC colony at the conclusion of the study.

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.

Daily urine samples will be collected from overnight trays, and water consumption will be monitored.

Other instructions for animal care staff: (check applicable entries)

Sick Animals

[X] Call Investigator

[ ] Clinician to treat

[ ] Terminate

[ ] Necropsy

Dead Animals

[X] Call Investigator

[ ] Save for Investigator

[ ] Bag for disposal

[ ] Necropsy

Pest Control

[X] Call Investigator

[ ] OK to use pesticides

[ ] No Pesticides in animal area

Hazardous Materials (only if in the animal room):

Infectious Agents? [ ] Yes [X] No

Agent(s):

Radioisotopes? [ ] Yes [X] No

Agent(s):

Chemical Carcinogens? [ ] Yes [X] No

Agent(s):

Toxic Chemicals? [X] Yes [ ] No

Agent(s): bromodichloromethane (BDCM)
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.

Chlorination of drinking water is used worldwide to prevent infectious diseases. However, the disinfection by-products generated from chlorination have a wide range of adverse effects on humans and laboratory animals. Trihalomethanes (THMs) are among the highest in concentration of these by-products. THMs are comprised of four compounds: chloroform, bromodichloromethane (BDCM), chlorodibromomethane (CDBM) and bromoform with chloroform and BDCM being the two most prevalent compounds. Humans are exposed to THMs daily through dermal absorption, inhalation and ingestion. It has been reported that potential toxic levels of THMs exist in municipal drinking water supply systems as well as in drinking water from water-vending machines in California. Because of the large daily consumption of water by humans, several recent reports and editorials have heightened public concern. Because water is such a vital issue in the western United States, it is likely that this topic will be of even larger public concern in the future. While most toxicological studies have focused on the carcinogenic potential of THMs, a relatively low association between human exposure to total trihalomethanes (TTHMs) in drinking water and the cancer risk has been minimized because these reports agree that the risk of cancer occurs at levels of exposure that are unlikely to occur via drinking water.

Only a few published studies have focused on the effects of THMs on reproductive outcomes and attention now needs to be focused on the adverse effects of THMs on reproductive health for two reasons. First, special attention to reproductive hazards is required because adverse effects on reproductive function, particularly pregnancy, are likely to occur at exposure levels that are lower than those that cause cancer. In addition, because reproductive health is not an immediate health concern and adverse effects on human reproduction can be concealed for many years, specific investigations must be initiated. The increased risk of small-for-gestational-age (SGA) birth, the elevated odds for stillbirth, spontaneous abortion, term low birth-weight among women exposed to TTHM have been reported and support the concept that THMs in drinking water is a reproductive hazard to human reproductive health. It is not clear which compounds are responsible or what concentrations should mandate concern.

The relative concentrations of individual THMs components in drinking water systems depend on variables primarily related to the water supply—such as pH, bromide ion concentration and temperature. According to an EPA report, guidelines are set only for total THMs (TTHMs) in drinking water systems and the concentration for individual compounds, such as
BDCM, may average levels of 12.7 ug/L with maximum concentrations reaching 183 ug/L. Among all published reports only two investigate the association of individual THM compound exposure and human reproductive outcomes. Waller and colleagues reported an increased risk of spontaneous abortion among women who drank 5 glasses per day of cold tap-water with 75 ug per liter total trihalomethane in California and stratified analysis further showed that only BDCM exposure was associated with spontaneous abortion. King et al. also reported the increased risk of stillbirth and THMs consumption in Canada and the strongest association was BDCM exposure, where risk doubled for those exposed to a level > 20 ug/L compared to those exposed to < 5ug/L. When the cause-of-death based on the physiologic processes responsible for the fetal death was investigated, relative risk estimates associated with BDCM exposures were larger for asphyxia-related deaths than for unexplained deaths or stillbirths.

Reproductive toxicity studies need to be continued because the mechanism of the adverse effect of BDCM on reproduction is only now being formulated in laboratory rodent models. While the rodent is clearly not an appropriate model for human pregnancy, the results of these studies clearly suggest the potential hazard to human reproductive health. Full-litter resorption as well as the decreased serum progesterone levels were observed in Fischer-344 rats following aqueous gavage treatment of BDCM between gestational days 6-10, a critical sensitive period for BDCM exposure during the luteinizing hormone-dependent period of pregnancy. Similar effects were not observed when BDCM was given on GD 10-15 and the authors speculated that BDCM affects the responsiveness of corpus luteum to pituitary luteinizing hormone (LH). However, a pituitary-dependent event does not occur during human pregnancy.

Although rodents have been used as a valuable model to study the potential adverse effects of environmental hazards on human reproductive outcomes, the physiological and reproductive differences between the two species are substantial. In higher primates, including humans, it is the product of conception (trophoblast cells) that supports the pregnancy. In contrast, the early rat pregnancy depends on pituitary LH under the control of the hypothalamus and higher brain centers. Pregnancy in higher primates is supported by an LH-like substance, chorionic gonadotrophin (CG), produced by the trophoblast cells and secreted autonomously into the mother's circulatory system. The ability of the placenta to produce CG is dependent on its ability to invade the uterine lining (endometrium) and differentiate into multinuclear synciotrophoblasts and grow into a relatively large organ. Rats neither produce CG during early pregnancy nor form an invasive synciotrophoblast or develop large placentae. In fact, the rat placenta is structurally quite different from that of the human. Therefore, the mechanism of BDCM to induced failure to maintain the pregnancy in rats is unlikely to reveal a mechanism that is relevant to human pregnancies and data extrapolation from rodents to humans may not be appropriate and could be misleading.

The results of et al. indicate a strong association with asphyxia-related human fetal deaths which is intriguing and provides an important clue. Asphyxia of the fetus spontaneously occurs as a result of premature separation of the placenta, which, in general, indicates the inability of the placenta to continue to supply the fetus with oxygen and other nutrients. This observation suggests a placental target of toxicity in the human rather than the ovarian target of toxicity that is suggested by the rodent data. It is likely therefore that the mechanism of toxicity in the human may be operating more slowly by reducing placental formation, development and function. In our recent in vitro study using human term trophoblast cells, we showed that BDCM treatment reduced CG production from syncytiotrophoblast cells (differentiated trophoblast cells). While this reduction of CG is statistically significant, it is unlikely to be...
sufficient to cause early pregnancy loss by itself but this observation does confirm that the trophoblast is a potential target of BDCM toxicity. This structural and hormonal compromise to the early trophoblast is consistent with later fetal loss and proof of concept. The use of the in vitro data to explain the epidemiological data is now biologically plausible because an exposure early in pregnancy, when the placenta is forming, is likely to ultimately affect the future growth and development of the placental structure and finally its ability to function. As the fetus grows, it will eventually outstrip the ability of a compromised placenta to provide oxygen and nutrients. When BDCM was administered to undifferentiated trophoblast cells in vitro, the cells were unable to differentiate into syncytiotrophoblast cells in differentiation-inducing medium. Trophoblast cells are the major cell type that develop into the functional placenta and are responsible for CG secretion and more importantly support of the fetus. Any disruption of the development or differentiation of these cells would result in placental insufficiency, one of the causes of fetal suffocation due to lack of oxygen and nutrients. Our in vitro data are consistent with this possibility and predict the outcome. Therefore, our in vitro results are consistent with the epidemiological findings and provide a potential explanation for BDCM induced fetal loss in humans. We hypothesize that early trophoblast cells are the targets of toxicity and adverse effects at this time lead to placental failure in later pregnancy.

The objectives of this study are to document the target of BDCM and provide insight into the mechanism of BDCM induced abortion. The results of this study will help public health agencies establish safety guidelines for individual components of THMs levels, specifically BDCM, in drinking water.

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.
- [x] catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [ ] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see i below)
- [ ] Trapping, banding or marking wild animals
- [ ] Special diets; food or water treatment.
- [ ] 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.)

In Group 1, thirty-five female cynomolgus macaques will be screened for normal menstrual cycles and reproductive activity. Daily urine samples (approximately 3 mL/day/animal) will be collected from cage pan for approximately 35 days. All animals will be time-mated every other day to proven males during cycle days 9 through 13. Urinary FSH will be evaluated during the breeding period immediately following cycle day 13 prior to treatment to identify animals that have ovulated. Only animals that have ovulated and have positive pairing (presence of the sperm in the vagina) during the fertile period (pairing from two days before through the day of the ovulation) will be candidates for treatment. Blood samples (2 mL/sample) will be drawn from arm or leg pull from animals that are briefly cage-restrained on days 8, 10, 12, 13, 14, 16, 18, 20, 22, 24, 26 and 28 days post ovulation. All mated animals will be monitored for chorionic gonadotropin and serial ultrasound to detect early pregnancy.
In Phase I, a dose finding study will be conducted. Group 2 animals (n=8) used in this phase will be a subset of Group 1. Since the toxicity of BDCM in primates has not been assessed and there are no preliminary data available, we have selected three initial doses of 150, 300 and 500 mg/kg based on rodent data. Initially, we will test the highest dose (500 mg/kg in corn oil) on a single pregnant monkey. Daily oral doses will be administered in the immediate post-implantation period from GD 12 through 18 (day of ovulation is designated as gestational day 0) once per day using naso-gastric intubation. Hysterotomy will be performed on GD 28 under ketamine and isofluorane anesthesia and the entire conceptus will be removed. Oxy-morphone will be administered (IM) for two to three days to relieve any post-surgical discomfort. Implantation site tissues will be collected and evaluated. If any maternal toxicity (hepatic, renal) is detected by clinical parameters (CBC, chemistry panel, liver enzymes & by-products, etc as recommended by CNPRC veterinarians) then the medium dose (300 mg/kg) will be tested in a second pregnant monkey. If maternal toxicity is detected at the medium dose, a third pregnant monkey will be given the 150 mg/kg dose. Conversely, in the event that no maternal toxicity or adverse placental effects are detected at 500 mg/kg, the dose will be increased by five-fold to 2500 mg/kg. This strategy will be employed until we find a dose that shows observable adverse effects on placenta without maternal toxicity. Our experimental dose (for Phase II, Group 3) will be the lowest dose which does not cause maternal toxicity but has adverse effects on the placenta. The data from each animal in this group will be evaluated prior to starting the next animal in order to reduce the number of animals used in this dose finding phase. Since BDCM toxicity has never been assessed in higher primates, it may be necessary to use up to 8 animals in this portion of the study to determine the appropriate dose. Once the target dose has been determined, this will be the dose administered to the animals in Group 3.

In Phase II, the target dose from phase I will be tested. Group 3 animals (n=4) in this phase will be a subset of Group 1. The target BDCM dose (in corn oil) will be administered daily to three pregnant monkeys in the immediate post-implantation period (GD 12-18) using naso-gastric intubation. On GD 28, hysterotomy will be conducted and implantation site tissues and the embryos collected as described in phase I. Group 4 animals (n=4) will serve as our control group. The corn oil vehicle (1 ml/kg) will be administered daily to three pregnant monkeys in the immediate post-implantation period (GD 12-18) using naso-gastric intubation. On GD 28, hysterotomy will be conducted and implantation site tissues and the embryos collected as described in phase I.

Animals that do not conceive will be returned to the study and mated again over no more than three consecutive menstrual cycles, at which time all non-pregnant animals will be removed from the study. It may be necessary to screen additional females if there is a low conception rate in the initial selection of animals. After the phase II portion of this study, all animals not used in this study will be returned to the colony. Study monkeys will be returned to the colony after hysterotomy.

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.

<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>Ovarian function monitoring, characterizing, timed mating, urine and blood collection.</td>
<td>35</td>
<td>1</td>
</tr>
</tbody>
</table>
2 (subset of group 1)
Target dose finding: BDCM (various doses in corn oil) administered via naso-gastric intubation (once per day on GD 12-18), blood and urine samples, hysterotomy for placenta and embryo removal.

3 (subset of group 1)
Experimental group: BDCM (target dose from group 2 in corn oil) administered via naso-gastric intubation (once per day on GD 12-18), blood and urine samples, hysterotomy for placenta and embryo removal.

4 (subset of group 1)
Control group: Corn oil (1 mL/kg) administered via naso-gastric intubation (once per day on GD 12-18), blood and urine samples, hysterotomy for placenta and embryo removal.

---

### 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 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 macaques are a well-established reproductive model for humans due to qualitative and quantitative similarities in menstrual cycle characteristics, hormonal profiles and reproductive anatomy. Since the toxicity of BDCM on primates has not been assessed and there are no preliminary data available, a dose finding study is necessary and may require the use of up to 8 animals to determine the appropriate target dose. Since data on each animal will be evaluated prior to starting the next animal, we hope to minimize the number of animals used in this phase of the study. The number of animals in the treated and control groups (n=4/group) is the minimum requirement for statistical analysis using ANOVA with repeated measures to evaluate the potential adverse effect of BDCM on early pregnancy.

Based on previous studies conducted at the CNPRC, the rate of conception is approximately 25% for normal cycling females bred over four consecutive menstrual cycles. In order to obtain enough pregnant females in a timely manner to conduct this study, we have set the minimum number of primates needed at 35.

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

Building: CNPRC Surgical Suite  
Room: 1316

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>Cynomolgus Macaques</td>
<td>Ketamine HCL</td>
<td>10</td>
<td>IM</td>
<td>1X/Hysterotomy</td>
</tr>
<tr>
<td></td>
<td>Atropine</td>
<td>0.04</td>
<td>IM</td>
<td>1X/Hysterotomy</td>
</tr>
<tr>
<td></td>
<td>Isofluorane</td>
<td>To effect</td>
<td>Inhalation</td>
<td>1X/Hysterotomy</td>
</tr>
<tr>
<td></td>
<td>Oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>1X/day for 3 days post-hysterotomy</td>
</tr>
</tbody>
</table>

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 on the study will ensure maintenance of health and animal well-being. The treatment regimen of BDCM will be selected to minimize the adverse effects. BDCM has not been studied in higher primates, therefore we are unsure of the side effects in monkeys. However, studies in rodents indicated hepatic and renal toxicity at very high doses and given over extended periods of time. Animals will be monitored for signs of liver, renal and hematological toxicity through out the administration of this compound. Mild discomfort may be experienced after hysterotomy. In the event of illness, animals will be treated by CNPRC veterinary staff.

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 adverse effects will be ameliorated or alleviated at the request of and under the guidance of CNPRC veterinarians including the use of analgesics to relieve post-surgical discomfort.

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:

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/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search? 02/12/03

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>Entrez</td>
<td>1974-2002</td>
<td>Same</td>
</tr>
<tr>
<td>ISI Web of Knowledge</td>
<td>1990 to 2002</td>
<td>Same</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

No alternative methodologies to animal use were found in published literature. However, our laboratory has conducted an in vitro study where BDCM was added to undifferentiated trophoblast cells and found that the cells were unable to differentiate into syncytiotrophoblast cells in differentiation-inducing medium (see section a). While our in vitro findings provide a potential explanation for BDCM induced fetal loss in humans and support our hypothesis that early trophoblast cells are the target of toxicity and adverse effects at this time lead to placental failure in later pregnancy, these findings alone do not provide sufficient evidence to prove that BDCM ingested via drinking water causes reproductive toxicity. An in vivo study is needed to determine the target of BDCM and its mechanism of toxicity. There are some published studies in various animal models on the carcinogenesis and reproductive toxicity of BDCM. However, to our knowledge, there are no published articles to evaluate the effect of BDCM on fetal loss in nonhuman primates, the only animal model in which toxic effects on early pregnancy can be used to produce data that can be interpreted in terms of human pregnancy.

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.

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

Euthanasia is not a part of this study, but will be used at the discretion of a senior CNPRC veterinarian.

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>Cynomolgus Monkeys</td>
<td>Overdose</td>
<td>Pentobarbital</td>
<td>60 mg/kg</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?

All animals will be returned to the colony at the conclusion 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.

<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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

PROTOCOL #_10496__
EXPIRES: __________

RUA#: __________
BUA#: __________
CCA#: __________

Identity of Hazard: Bromodichloromethane – toxic

Investigator Last Name: __________ Department: __________
First Name: __________ Phone: __________
Email: __________ Fax: __________

Provide a short description of the agent:
Bromodichloromethane (BDCM) is a drinking water disinfection by-product.

This agent / material is hazardous for: [ ] Humans only [ ] Animals only [X] Humans and Animals
For which Animal Species?

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

Describe any human health risk associated with this agent:
This compound is a respiratory system, skin and serious eye irritant.

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:
[X] The following personal protective equipment must be worn/used in the room:

[X] Lab Coat/Coveralls [X] Shoe Covers/Booties
[X] Disposable Gloves [X] Head Cover
[X] NIOSH Certified Dust Mask [ ] Disinfectant footbath
[X] Eye Protection/Face Shield [ ]

[ ] Fitted Respirator Type:
[ ] Other: Describe:

[X] Personal protective equipment must be removed before leaving the room.
[X] Personal protective equipment must be discarded or decontaminated at the end of the project
[X] Hands, arms, and face must be thoroughly washed upon leaving the room
[X] Full shower, including washing of hair, must be taken upon leaving the room.
[X] 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: