PROTOCOL FOR ANIMAL USE AND CARE

Investigator:

Last Name: ___________________________  First: ___________________________  Middle: ___________________________

Last Name: ___________________________  First: ___________________________  Middle: ___________________________

email: ___________________________  email: ___________________________

Department: ___________________________

Department: ___________________________

Phone / Fax: ___________________________

Phone: ___________________________

After hrs. #: ___________________________

After hrs. #: ___________________________

CNPRC

PROTOCOL: 10814

EXPIRES: 10814

EH&S USE ONLY

Email to: campusvet@ucdavis.edu

Species (common names):  Number:  Source:

Cynomolgus Macaque  18  CNPRC breeding Colony

Cynomolgus Macaque  12  Offspring produced from study

Project Title: TCDD 09: Biomarkers for Reproductive Toxicity in Non-Human Primates

Overnight housing location: CNPRC  Day use: 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.

The objective of this study is to detect and characterize the developmental changes associated with orally dosed TCDD (Dioxin, 0.5 µg/kg BW) from the immediate post-implantation period onward. Pregnancies will be closely monitored and selected urine and periodic blood samples will be collected throughout pregnancy. Offspring (between 3-4 months old) will be evaluated for social and behavior traits.

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.

Metabolism cages for animals treated with TCDD (Dioxin).

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

Sick Animals

[x] Call Investigator  [ ] Call Investigator  [x] Call Investigator

[ ] Clinician to treat  [ ] Save for Investigator  [ ] OK to use pesticides

[ ] Terminate  [ ] Bag for disposal  [ ] No Pesticides in animal area

[ ] Necropsy  [ ] Necropsy

Dead Animals

Pest Control

Hazardous Materials (only if in the animal room):

Infectious Agents? [ ] Yes  [x] No  Agent(s): [ ]

Radioisotopes? [ ] Yes  [x] No  Agent(s): [ ]

Chemical Carcinogens? [x] Yes  [ ] No  Agent(s): TCDD

Toxic Chemicals? [x] Yes  [ ] No  Agent(s): TCDD

University of California, Davis
Printed 7/21/2004 10:50:32 AM  Page 1
Funding source: NIEHS
Previously approved? [x] Yes [ ] No
Previous protocol number (if any): 9226

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

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

Our most recent experiment revealed subtle structural defects in the neural cord of the embryo following a single exposure of the mother to TCDD. We hypothesize that such defects would be permanent but not lethal and may result in learning performance and social skills in the neonate similar to autism. The current study will determine if such defects are a result of low-dose chronic exposure and if so, determine their severity.

Although the cause of many developmental defects is known to be attributable to specific environmental exposures, many have no known pathogenesis. The occurrence of autism in the Central Valley, for instance, has increased almost 300% over the past 20 years and has no genetic or exposure link. An environmental link to autism would provide a major breakthrough in this field. Unfortunately, early pregnancy exposures are extremely difficult to document and there are no non-primate species that develop similar to humans. TCDD (dioxin) is the most potent member of a group of environmental hazards, arylhydrocarbons (Ah). These compounds are relatively common in our daily lives found in soil, manufactured items and food. Perhaps one-half of the human exposure to Ah is produced by combustion including forest fires. It is not impossible that such compounds could be responsible for a portion of the unexplained rise birth defects including autism.

Over the past ten years we have been successful in documenting the adverse effects of Ah in the nonhuman primate animal model with exposures in the immediate post-implantation period. We have established that 1-2 µg/Kg BW TCDD is not embryo lethal and marginally toxic to the dam. We have employed relatively high dose acute exposures (4 µg/KG TCDD BW) in our most recent studies and revealed neurotube defects. One unexpected finding of these studies was the suppression of specific circulating fatty acids that are critical building blocks for embryonic nerve tissues. This TCDD-induced depression of key fatty acids and its association with subtle alterations in the structure neural cord of the embryo drive the current experiment. We speculate that Ah-induced defects could lead to functional defects in the neonate. The goal of this study is to characterize the developmental changes that are associated with TCDD-induced changes in neural development. In the proposed continuation of this protocol we will reduce the exposure dose to 0.5 µg/KG BW once a week but extend the exposure period to eight weeks in order to induce neural development defects without causing maternal toxicity or embryonic death. At 3-4 months of age, the infants will be evaluated by a behaviorist using a well-defined battery of tests.
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
- Food or water restriction
- Special diets; food or water treatment.
- Polyclonal Antibody Production
- Non-recovery surgical procedures
- Induced illness, intoxication, or disease
- LD 50 or ID50 studies.
- Survival surgical procedures
- Death as an endpoint (see i below)
- catheters, blood collection, intubation
- Multiple survival surgery
- Trapping, banding or marking wild animals
- Fasting prior to a procedure.
- Behavioral modification.
- Aversive conditioning.
- Prolonged restraint. (8 hrs+)
- Multiple survival surgery
- Death as an endpoint (see i below)

** 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.)

**OVERVIEW:** In the proposed study, 18 animals (group 1) will be prescreened for normal ovarian function using daily urine sampling and their menstrual calendars. Normal animals will be timed-mated at the next fertile period. The first 12 animals (groups 2 and 3) determined to be pregnant will enter the protocol. Non-pregnant animals will be rebred twice before being removed from the study. Once twelve animals are diagnosed and pregnant and reach the third week of pregnancy all breeding will stop. Six (group 2) of the pregnant animals will receive 0.5 µg/Kg BW TCDD at a volume of 1.0 ml/KG BW of corn oil/dioxane 9:1 v/v once each week via nasogastric lavage for eight weeks starting on GD 16. Six animals (group 3) will receive the vehicle only and monitored identically to the treated animals. Animals will be hand-caught from their respective cage and hand-restrained briefly (approximately 2-3 minutes) for treatments. All pregnant animals will be permitted to produce live neonates (group 4) which will be undergo biobehavioral testing between the ages of 3-4 months. Loss of pregnancy prior to term will result in a complete autopsy of the fetus with special emphasis on neural development.

**SAMPLE COLLECTION:** Daily urine samples will be collected from cage pans and analyzed for ovarian and placental hormones one month prior to and one month following the diagnosis of pregnancy. One blood sample (2ml) on early follicular phase (i.e. 3-5 days after the beginning of menses) will be obtained from all animals (group 1). Pregnancy detection (group 1 animals) will consist of serial ultrasound exams (no more than 3 times per week) starting 1 week post confirmed mating and confirmed by serum monkey chorionic gonadotropin (mCG) determinations through first month of pregnancy or until non-pregnancy is confirmed. Blood samples will be collected every other day starting on GD 14 and continue until GD 28 (8, 2 mL samples). From the second month of pregnancy through term (group 2 and 3), or loss of pregnancy, monthly ultrasound exams will be performed, as well as a monthly blood sample (5 ml). Ultrasound exams will be done on morning fasted animals while animal is briefly anesthetized. Blood will be collected from arm-pull of cage-restrained animal from cephalic vein. General health and progression of the pregnancy will be closely monitored (body weight, ultrasound, serology).

**SAMPLE ANALYSIS:** All urine samples will be analyzed for the metabolites of estradiol and progesterone and will be used to compare ovarian function and function of the feto-placental unit between treated and control animals. The initial blood sample from the early follicular phase will be analyzed for lipids, and the following samples in early pregnancy (GD 16-28) will be analyzed for mCG, TCDD and lipids. The monthly blood samples will be split and 1.0 mL used immediately for serology and the remainder archived for...
future evaluation of fatty acids and other analytes as indicated by the study outcome. The urine samples will be used to measure estrogen metabolites.

BEHAVIORAL TESTING OF INFANTS (Group 4):

Offspring will be tested during 3 to 4 months of age. Each monkey will be tested for a 24-hr period, and all testing will take place during May through October.

Three-to-four-month-old cynomolgus monkeys and their mothers will be relocated from their living cages to individual holding cages. Mothers will be immobilized with ketamine (10mg/kg), and infants will be removed and taken to a testing/housing area. Mothers will remain in the holding cages. Infants will be housed individually with ad lib access to food, and a variety of novel objects and towels in their cages. During the 24-hr period, animals will be assessed behaviorally and physiologically in a number of standardized situations to identify individual differences in biobehavioral organization:

1. Focal observations (DAY 1). Each animal will be observed unobtrusively for 5 min. in its housing cage. Principal measures will be activity, vocalization, and contact with objects, and the goal is to determine individual differences in response to the initial separation from mother and relocation to a novel setting.

2. Blood sampling (DAY 1). Animals will be physically restrained briefly and a 1-ml sample of blood will be drawn. Blood will be assayed for numbers of CD4+ and CD8+ T-cells (the ratio of which has been shown to be trait-like and under genetic control) and cortisol, a stress-related hormone. Cortisol responses to separation and relocation have also been shown to be trait-like.

3. Responsiveness to human threat (DAY 1). Using a procedure used in some of the behavioral studies at the Primate Center (and those conducted in other laboratories), animals’ responses to a human displaying a profile or full frontal face will be recorded. Responses of interest include positional, activity, and emotional behaviors. Responses to this test have been shown to be stable over time (ie, reflect stable, individual differences), and are related to frontal EEG activity, and certain personality variables.

4. Social responsiveness (DAY 1). Using a videotape playback paradigm, animals will be exposed individually to a 10-min videotape of an unfamiliar animal displaying aggressive behavior. Responses of interest include positional and social behavior, and have been related to the major personality dimensions Sociability and Confidence.

5. Recognition memory (DAY 1). Each monkey will be tested using a paired comparison task. Each animal will receive 10 trials. On each trial the animal will be shown two identical stimuli (objects or pictures), followed by a brief interval, after which the familiar and a novel object is displayed. The principal measure of interest is duration of looking at the novel object. This test reflects visual recognition memory, and performance has been related to later cognitive impairment, and developmental problems.

6. Pituitary-adrenal regulation (DAY 1-2). Animals will be physically restrained briefly and a 0.5-ml sample of blood will be drawn for assay of cortisol. Each animal will then be injected with 500ug/kg of dexamethasone. Dex is a synthetic glucocorticoid that can suppress endogenous cortisol output. The next morning, a 0.5-ml blood sample will be taken to determine the efficacy of Dex in suppressing cortisol. Failure to suppress cortisol in humans is associated with negative affective personality characteristics (esp. depression). Finally, each animal will be injected with 2.5 IU ACTH in order to stimulate cortisol output. Thirty minutes later, a final 0.5-ml blood sample will be drawn for assay of cortisol. The dex and ACTH stimulation tests are clinical tests used routinely to determine the integrity of the hypothalamic-pituitary-adrenal system. We have data
suggesting that the response of the HPA system is traitlike – individual differences in cortisol concentrations (including responses to dex suppression) are maintained over time. Because of the interrelations of the HPA and immune system, understanding of the regulation of the HPA system may provide information on which animals are at greater risk for poor health outcomes.

7. Focal observations (DAY 2). Each animal will be observed unobtrusively for 5 min. in its housing cage. Principal measures will be activity, vocalization, and contact with objects. Comparison of the Day 2 with Day 1 focal observations will provide information on individual differences in adaptability to separation and relocation. At approximately 10 AM, infants will be reunited with their mothers in the mothers’ holding cages. Prior to the reunion, mothers’ temperament will be assessed by having a trained technician hand-present three preferred food items, and record the mothers’ behavioral responses. Following the reunion, mothers and infants will be observed for approximately 1 hr. to insure that the mothers will accept the infants, and to allow an opportunity for the infants to suckle. Mother-infant pairs will then be returned to their original living cages. Observations will continue to insure that the reintroductions to the cages occur safely.

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>Screening and breeding</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.5 µg/KG/TCDD/week X eight weeks at (0.5 µg/1.0 ml)</td>
<td>6 (subset of group 1)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle: corn oil:dioxane (9:1) control at 1.0 ml/KG BW/week X eight weeks</td>
<td>6 (subset of group 1)</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Offspring (at 3-4 months of age will be studied for biobehavioral testing under AUC #10365)</td>
<td>12 (produced from treated and control group animals)</td>
<td>3 (Maternal deprivation for 24hrs)</td>
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</table>
### Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
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</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?

The rationale for the experimental design is based on the results of our previous studies with groups of 4 to six animals. We have successfully treated pregnant monkeys with single TCDD doses so low that they cause no detectable effect (1 µg/Kg BW) to doses that induce of early abortion and in one instance the death of the mother (4 µg/Kg BW). In the present study we will use a very low, non-toxic dose but administer it repetitively over two months. We believe this dose will not have adverse effects on general metabolism but will affect development of the embryo. Since the monkey is the only animal model which has embryonic and fetal development similar to that of the human, we have no alternative choice of animal model. We believe that six treated animals is the smallest number that can be used to produce a reliable report.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
<th>Surgeon</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Center Vet Staff</td>
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</table>

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>M. fascicularis</td>
<td>Ketamine HCl</td>
<td>10</td>
<td>IM</td>
<td>1 time/serial ultrasound</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 this study will ensure maintenance of health and animal well-being as it is possible that TCDD-induced abortion could occur. The treatment regimen for TCDD was selected to minimize adverse effects and we expect none. Previous study animals experienced no side effects at the dose proposed in this study. Various side effects that were seen previously in a few of the animals that received higher doses (4, 6, and 8 times proposed individual doses) included alopecia, dermatitis, facial swelling, partial or complete sloughing of fingernails and various changes in the eyes and eyelids. The side effects associated with the eyes/eyelids included swelling, excessive tearing, ocular discharge and loss of eyelashes. Because TCDD is rapidly sequestered in adipose tissue, we believe circulating levels will remain below toxic levels. None the less, animals will be monitored for signs of hepato-, nephro- and hematologic toxicity as well as monitored for fetal distress and impending abortion. In the event of illness, animals will be appropriately treated by our veterinary staff. In case of abortion the products of conception will be evaluated histologically.

In the biobehavioral portion of the study, previous studies using the behavioral protocol have shown that the infants display distress upon separation from their mother. As well, the infants may have difficulty adjusting to solid food.

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 with analgesics administered at the request and guidance of the veterinarian. The analgesics used will either be Ketoprofen at 2 mg/kg, IM, SID; Ibuprofen at 7 mg/kg, PO, BID; Tylenol at 6 mg/kg, PO, TID or Oxymorphone, 0.15 mg/kg, IM, SID.

The separation period between mom’s and their babies will last only for 24 hrs. In addition, a towel will be provided to the infants for contact comfort. Continuously available highly-palatable food will be provided to infants. Should an animal refuse to eat, it will receive subcutaneous fluids at the end of Day 1, when it receives the dexamethasone injection. Finally, we will provide a 1-hr period during reunion with mother for the infant to suckle, prior to the mother and infant being returned to their group.

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_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?  8-29-2003

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>
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<tbody>
<tr>
<td>CDL Current</td>
<td>93 (wk 27) - 2003 (wk 36)</td>
<td>Dioxin, primate, reproductive and developmental toxicity</td>
</tr>
<tr>
<td>Pubmed</td>
<td>1966-2003</td>
<td>Dioxin, Reproduction, toxicology</td>
</tr>
<tr>
<td>Entrez</td>
<td>1975-2003</td>
<td>Dioxin, Reproduction, toxicology</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

Our group is the major contributor to the literature in this area. We have not found any other publications that involve TCDD or related compounds administered to the non-human primate model or an investigation of pregnancy outcome with related drugs at the levels proposed in this study. There is no other animal model for this kind of experiment. There are similar experiments reported for rodent and avian species but neither of these models shares the specific qualities of implantation and embryonic development with the human. Results of experiments in rodents do not provide information that is relevant to neural development and neonatal defects in behavior, socialization and learning.

Has this study been previously conducted?  [x] Yes  [ ] No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

In previous experiments we used higher doses of TCDD to investigate the mechanism of toxicant-induced pregnancy loss. In the current experimental design we will maintain the pregnancy in order to investigate developmental defects in the neonate.

k) Disposition of animals:  At what point in the study, if any, will the animals be euthanized?
Euthanasia is not planned for this study but will be at the discretion of the attending veterinarian. At the end of the study the surviving treated animals and three of the controls will be assigned to a terminal study which will be explained in detail in a following application.

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>
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<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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<tr>
<td>M. Fascicularis</td>
<td>Barbituate overdose</td>
<td>Pentobarbital</td>
<td>60 mg/Kg</td>
<td>IV</td>
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m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project?

Because the treated animals will not be useful for any additional studies they cannot be returned to the colony. All surviving treated animals and three controls will be used in a terminal study after this study has been completed. TCDD has a disappearance rate of months to years and adverse effects have been observed as long as two years following a single exposure.

The other three controls not used for the next study as well as any offspring will be returned to the CNPRC 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.

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<th>Last Name</th>
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<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://ehs.ucdavis.edu/animal/health/](http://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at [http://ehs.ucdavis.edu/](http://ehs.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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
</table>

Committee Use Only Below

** Conditions necessary for Committee Approval:

<table>
<thead>
<tr>
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</table>

Final Disposition of this protocol:

________ Approved

________ Not Approved

________ Withdrawn by Investigator

Date of Action: _____ / _____ / _____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

<table>
<thead>
<tr>
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Campus Veterinarian

Date

University of California, Davis
ANIMAL ROOM SAFETY INFORMATION

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

Identity of Hazard: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Investigator Last Name: [ ]
First Name: [ ]
Email: [ ]
Fax: [ ]

Provide a short description of the agent:

chlorinated, polycyclic aromatic

This agent / material is hazardous for: [ ] Humans only [ ] Animals only [ x ] Humans and Animals

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:

We expect no hazards to humans under the protocol.

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.
[ x ] 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:

[ 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.

[ ] 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:

Concentration of TCDD administered to animals is within limits of safe disposal, however, the following precautions should be taken:
1) Animals should be housed in metabolism cages and wasted disposed according to CNPRC guidelines

2) Solid animal waste (feces, hair) will be bagged in the animal room and disposed of weekly by autoclaving prior to disposal. The state limit for disposal of TCDD is 10 ug/Kg and since each animal will receive only 4 ug, and most will remain in the animal for months, the waste will be below State requirements.

3) Liquid waste (urine and cage washing solution) will be collected into 5 gal drum located in the room in which animals are housed. Based on one-time, maximum dose of 4 µg/kg, and a maximum weight of 4.5 kg, it is estimated that the concentration of the urine/cage washing solution will be 1.0 µg/liter (= 18.0 µg/animal divided by 18.9 liter waste jug). This estimate is based on 100% excretion of TCDD and maximal animal weights and 4 µg/kg dose concentrations that will be used in the study. The limit for liquid waste is 1ug/L, so this protocol is within the limits.
08/27/03
Pre review questions

Hi,

I have received and pre reviewed the recently submitted protocol, which has been assigned accession number 10814 for future reference. I have attached a copy of the protocol so revisions can be made on the embedded copy.

For this protocol to be considered on the meeting agenda of September 11th, please forward you revised protocol to me on or before noon Tuesday, September 2nd.

Thanks in advance,

Protocol 10814 ( )
1. In section a, you refer to testing the behavior of neonates using a well-defined battery of tests. Please explain what the neonates will be subjected to in section c. Note: section c asks to you describe ALL procedures performed on the different groups of animals.

2. In section c, OVERVIEW, you mention that six animals will receive the vehicle only. What volume and what is the vehicle? How will the animals be restrained? Anesthetized or hand held and if anesthetized, will they be fasted? Please clarify.

3. You go on to briefly mention that the neonates will be studied under the standard CNPRC behavior battery of tests, but you will need to summarize what is happening. Please expand this section as one sentence is too brief. How long will the tests last and how often? Then what will happen to the off spring?

4. Under the sample collection section, you mention ultrasound, but have not included whether there is fasting or anesthesia involved Please clarify. When the blood is collected, will you use an arm or leg pull or anesthesia and then what vessel? Please clarify.

5. In section i, you state that in case of abortion, the products of conception will be collected for intensive study. What do you mean by this statement? What will you be looking for? Please expand.

6. You go on to mention the use of analgesics, but have not listed any in section g. Please expand to include analgesic agents, dose, route and frequency of administration.

7. In section j, you are asked to provide more than one database. You have just included one and have your last search date as 1/15/03. Please include an updated search date and an additional database searched.

8. On the animal room safety information sheet, you have not addressed the contaminated waste issue, yet you are having the solid waste collected. Please review this page for completeness.

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09/22/03
Pre review questions protocol 10814 from

Hi,

I have received the following committee question regarding the protocol 10814 on this weeks committee agenda. Please forward this question to Dr. and request a response on or before noon Wednesday. Please forward all correspondence to campusvet@ucdavis.edu.

Thank you in advance,
#10814
The investigator states that a behaviorist will be conducting the behavioral testing, yet this person has not been identified. Please include information on who or what behavior group will be conducting the testing.