

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

EH&amp;S USE ONLY

**PROTOCOL # 9772****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:	Total (3 years):	Source:
Rat (dams and offspring)	224	672	Zivic-Miller
Mouse (dams and offspring)	224	672	Jackson Lab
Hamster (dams and offspring)	60	180	Charles River
Guinea Pig (dams and offspring)	48	144	Charles River
Monkey (dams and offspring)	16	48	CRPRC

<b>Project Title</b>	Environmental Tobacco Smoke and Newborn Lung Development.		
Overnight housing location:	ITEH / CRPRC	Day use only:	
Animals will be maintained by:	<input type="checkbox"/> Vivarium <input checked="" 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.

Rats, mice, hamsters, and guinea pigs will be exposed to environmental tobacco smoke (ETS) generated by cigarette smoking machines in small animal exposure chambers located at the Institute of Toxicology and Environmental Health (ITEH). Animals will be exposed to ETS for 6 hours per day for up to 100 days. Monkeys will be exposed to ETS in large animal exposure chambers located at the California Regional Primate Research Center for 6 hours per day for up to 120 days.

**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.

N/A.

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

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

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

Infectious Agents?	<input 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:	UC Tobacco-Related Disease Research Program	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous Protocol No. (if any):	8346

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

<input checked="" 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 effects of exposure to well-characterized environmental tobacco smoke (ETS) during prenatal and perinatal lung development in rodents and monkeys will be examined. The hypothesis to be tested is that ETS impairs the lung maturational process in the prenatal and perinatal mouse, rat, hamster, guinea pig, and monkey. Pregnant mothers will be exposed to ETS for six hours per day by whole body exposure from gestation day 3 to 18 (hamster), gestational day 3 to 20 (mice), gestational day 3 to 21 (rats) or from gestational day 45 to 2 months postnatal age (monkeys). Experiments for guinea pigs will use a different exposure regimen with animals shipped from the supplier to UC Davis mid-gestation (approximately 30 days of gestation age). Exposure of timed pregnant guinea pigs will be from gestational day 35 to 60-62 days gestation (total gestational time is 63 days). Other guinea pigs will be studied only during the postnatal period beginning at postnatal day 8. Since guinea pigs are fully mature at birth and do not require maternal care, they can be shipped to UCD from the supplier 3 to 5 days after birth. Monkeys are also fully mature at birth, but will be maintained with their mother at all times during the course of postnatal exposures to cigarette smoke. ETS is defined as the combination of sidestream smoke released from the burning end of a cigarette and that portion of mainstream smoke exhaled by smokers. The effects of in utero exposure to ETS will be examined in newborn and 21 day old animals. Other experiments will examine the effects of ETS exposure in the postnatal rodent by whole body exposure to ETS from birth to 120 days of life. Exposure to ETS will be for a total of 6 hours per day.

**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 checked="" type="checkbox"/> Non-recovery surgical procedures	<input type="checkbox"/> Induced illness, intoxication, or disease
<input type="checkbox"/> LD 50 or ID50 studies.	<input type="checkbox"/> Survival surgical procedures	<input type="checkbox"/> Death as an endpoint (see i below)
<input type="checkbox"/> Catheters, blood collection, intubation	<input type="checkbox"/> Multiple survival surgery	<input type="checkbox"/> Trapping, banding or marking wild animals
<input type="checkbox"/> Prolonged restraint. (8 hrs+)	<input type="checkbox"/> Behavioral modification.	<input type="checkbox"/>
<input 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.)

All rodents will be housed in polycarbonate shoebox type cages with wire tops. Timed pregnant animals (hamsters, mice, and rats) will be housed 2 per cage through gestation day 18 (hamsters), 20 (mice) or 21 (rats) and then housed singly to allow for birthing. Timed pregnant guinea pigs will be housed singly from gestation day 30 (arrival from supplier) through term (~ 63 days gestation). Timed pregnant monkeys will be housed in one cage but there will be two monkeys housed per exposure chamber during the course of the experiment. Newborn guinea pigs will be housed with the mother for the first 3 days of life and will subsequently be housed in pairs without the mother thereafter. For experiments with monkeys, the mother will be maintained with the infant. Monkeys will be maintained in exposure chambers during gestation and post-partum. Fetuses will be sonographically assessed prior to exposure of the dams using standard techniques, then on approximately gestational day (GD) 120 and 150. Since animals will be delivering spontaneously, additional examinations may be included prior to delivery. Using established ultrasound-guided techniques, amniotic fluid (~1 ml) may be collected prior to exposure, then on approximately GD 120 and 150. Once the animals deliver, infants will be removed from the dams (approximately day 3-5 after birth) from 1-4 times for neurobehavioral and morphometric assessments (assess development of simple reflexes, motor skills, and growth). Animals will be exposed only to air or to diluted and aged sidestream cigarette smoke up to 120 days of age. Although work done with hamsters, guinea pigs, and monkeys will be less frequent, exposure will be at the same concentration as for mice and rats. No other physical chemical or biological agents other than cigarette smoke will be used in these experiments.

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.

For aging studies, 20 timed pregnant animals are used, based on the estimation of 10 pups per litter. This allows for 10 litters in the control group and 10 litters in the treatment group.

Based on 204 animals/species/year (plus 20 dams), the proportion of animals to be used for each procedure is:

#### Mice and Rats (Dams = 20, Offspring = 204)

Age (days)	Procedure	Biochemistry	Histology	Physiology	Category
1		30	12		1
21		30	12		1
50		24	12	24	1
100		24	12	24	1

The mothers (n=20) will not be considered part of the study and at the end of the exposure period will be sent to the Raptor Center. These numbers are required to obtain statistical significance in our analysis of the effects of ETS on lung growth and development during the first 100 days of life. For biochemical studies, 10 mice-rats/experiment will be used at 1 and 21 days of age due to the small size of the lungs at this age. At 50 and 100 days of age, 8 mice-rats/experiment will be used. For histology, 4 mice-rats/experiment will be used. For physiology, 8 mice-rats/experiment will be used. A total of 3 experiments will be done each year. These experiments reflect 3 critical periods of exposure to be used in mice and rats: 1) in utero exposure only, 2) postnatal exposure only and 3) in utero and postnatal exposure. These studies are essential in establishing critical windows of exposure during perinatal development that will be established only using mice and rats. Studies with hamsters and guinea pigs will be more limited (only one experimental protocol will be followed, based on our findings in mice and rats, but are critical to establish the effects of ETS on lung development to better extrapolate our findings to humans. The number of animals and studies to be done are listed below:

#### Hamsters (Dams = 12, Offspring = 48)

Age (days)	Procedure	Biochemistry	Histology	Physiology	Category
1		8	8		1
21		8	8		1
50		8	8		1

#### Guinea Pigs (Dams = 16, Offspring = 32)

Age (days)	Procedure	Biochemistry	Histology	Physiology	Category
1		8	8		1
50		8	8		1

The mothers (n=8 for hamsters and n=8 for guinea pigs) will not be considered part of the study and at the end of the exposure period will be sent to the Raptor Center. Studies to be done on hamsters and guinea pigs would involve fewer animals and be

done less frequently (compared to mice and rats). Our protocol would involve 8 timed-pregnant animals. Exposure conditions would be identical, with the exception of onset and duration of exposure. Since smaller litter sizes are typical of hamsters (i.e. 4 pups/litter) and guinea pigs (i.e. 2 pups/litter), studies would be limited to few ages and types of studies. We anticipate based on the number of young hamsters required that each mother will have an average of 4 pups. One pup from each litter would be used at 1, 21 and 50 days postnatal age for biochemistry and one pup/litter for histology. Each age will have a control group (n=4) and a treated group (n=4). For histological analysis, a minimum of 4 animals are needed/group/age (control and treated groups) for morphometric studies. These same animals will be used for immunohistochemistry and in situ hybridization. Biochemical studies require a minimum of 4 animals/group/age (control and treated). For guinea pigs, we anticipate each mother will have an average of 2 pups. One pup from each litter will be studied at 1 and 50 days of age. Physiological measurements completed in guinea pigs at 50 days of postnatal age will use the same animals found in the histology category.

### Monkeys (Dams = 8, Offspring = 8)

<u>Age ((weeks)</u>	<u>Procedure</u>	<u>Biochemistry</u>	<u>Histology</u>	<u>Physiology</u>	<u>Category</u>
1-6		8			1

The mothers (n=8) will not be considered part of the study and at the end of the exposure period will be returned to the Colony at the Primate Center. Studies to be done on monkeys will involve the fewest number of animals (i.e. 8 per year). In each instance, tissues from each monkey will be used for each procedure (i.e. biochemistry, histology, and physiology), therefore animals are only listed under the biochemistry category above, but these same animals will be used for histology and physiology. Only the infant monkey will be taken in these experiments. The mother will be returned to the colony. These same mothers may be used in subsequent experiments if they continue to be used in the colony breeding program.

The emphasis of our research will be directed towards mice and rats due to the high reproducibility in obtaining timed pregnant animals and large litter sizes in contrast to hamsters, guinea pigs, and monkeys. Based on the small size of the lungs in mice and rats, greater numbers of animals are required. Only confirmatory studies will be done in hamsters, guinea pigs, and monkeys with fewer numbers of animals.

For all rodent species (mice, rats, hamsters, and guinea pigs), separate animals will be used for physiological and histological studies due to the small size of the lungs and subtle but significant alterations in the anatomical structure of the lungs following physiological maneuvers. Since the lungs from monkeys are larger, the lungs can be divided into lobes and individual lobe from the same monkey can be used for biochemistry, histology, and physiology.

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?

The species selected for this project are ideal to investigate the mechanisms of ETS effects in the perinatal period life and will allow us to extrapolate our findings to humans. Mice, rats, hamsters and guinea pigs are ideal species for our studies due to their size and relatively short gestational period (mice, rats, and hamsters) and rapid period of postnatal development. Mice and rats have a longer postnatal period of development in contrast to hamsters. These differences can be exploited to better define the effects of pre and postnatal exposures to ETS in species with different patterns of lung development and cellular differentiation. Guinea pigs with a much longer gestational period (63 days) will allow us to examine the effects of a longer period of maternal ETS exposure on fetal development that is more relevant to the potential effects noted in humans. Monkeys represent perhaps the closest match to the human in terms of lung development during the perinatal period. Although the development pattern of the lungs in rodents is similar to that seen in humans, they all have a greatly condensed period of time during gestation. The size of rodents is also ideal for our research due to housing limitations within the exposure chambers.

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

Building:

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?

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)

No pain or discomfort of the animals is anticipated. The animals will be exposed only to sidestream cigarette smoke under conditions that would be equivalent to being in a very smoky room. We have noted in earlier experiments a slight reduction in body weight (5%) compared with controls, but all animals appear healthy. Animals will be provided food and water ad libitum during the course of the study. Since we have had no problems of stress, fetal resorption or spontaneous abortion in timed pregnant rats exposed to ETS, we assume the same will be true with mice, hamsters, guinea pigs and monkeys. We have also initiated exposure of pregnant guinea pigs at a later gestational period to minimize the risk of spontaneous fetal resorption during the first half of gestation. Although not anticipated under the conditions used in our studies, evidence for spontaneous resorption of fetuses for all species will be examined. No stress due to exposure conditions or timing of exposure is expected based on our extensive past experience with mice and rats. Should such problems arise with our limited use of hamster, guinea pigs and monkeys, we will consult with the attending veterinarian and ARS veterinary services for advice and further guidance.

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.

*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 our studies. All animals will be euthanized at the end of the designated period of exposure.

**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?

September 2001

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
Melvyl Medline	1990-2001	Environmental tobacco smoke
Current Contents	1990-2001	Environmental tobacco smoke
Pub Med	2000-2001	Environmental tobacco smoke

What were your findings with respect to alternative methodologies?

These studies are based on the analysis of the effects of exposure to environmental tobacco smoke on lung growth and development which require exposure by inhalation. Other routes of administration would not be appropriate.

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.

We have conducted cigarette smoke exposure experiments over the past 7 years and extensively published the results of our findings. Our work has generated new questions which we are currently pursuing through the support of EPA and the UCTRDRP.

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

At the end of the experiment, the animals will be euthanized. Since all experiments are terminal, animals are killed under anesthesia without regaining consciousness.

**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
Rat	Overdose	Pentobarbital	165 mg/kg	I.P.
Mice	Overdose	Pentobarbital	165 mg/kg	I.P.
Hamster	Overdose	Pentobarbital	165 mg/kg	I.P.
Guinea Pig	Overdose	Pentobarbital	165 mg/kg	I.P.
Monkey	Overdose	Pentobarbital	60 mg/kg	I.V.

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

Rodents will be donated to the Raptor Center or shared with other investigators involved in research with environmental tobacco smoke who have approved protocols to conduct their experimental studies.



