

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

EH&amp;S USE ONLY

**PROTOCOL # 9996****EXPIRES: \_\_\_\_\_****Investigator**

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
Fax:	

**Contact**

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
Fax:	

<b>Species (common names):</b>	<b>Number:</b>	<b>Source:</b>
Rhesus Monkeys	56	CRPRC

**Project Title** Pulmonary Effects of Environmental Pollutants in young monkeys.

Overnight housing location:	CRPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

**Procedures:** Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Neonate monkeys that are nursery housed and sensitized to house dust mite antigen on days 2-7 days after birth will be exposed starting at 2-4 weeks of age to repeated episodes of aerosolized house dust mite antigen and/or the photochemical air pollutant ozone in order to determine the role that ozone plays in exacerbating allergic asthma and lung development.

**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.

Animals must remain free from infectious respiratory disease.

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

<b>Sick Animals</b>	<b>Dead Animals</b>	<b>Pest Control</b>
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input checked="" 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: NIEHS and CRPRC Base Grant

Previously approved?  Yes  NoIs the project already funded?  Yes  No

Previous protocol number (if any): 8533

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

If you checked "Another Veterinarian", please provide:

Veterinarian:

Address:

Day phone:

Email:

Emergency phone:

*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.

This series of experiments will test the overall hypothesis that oxidant stress from episodes of exposure to ozone retards the structural and immunological development of the respiratory tract in young animals and exacerbates the development of asthma when allergen exposure occurs during the portion of the episode where acute inflammation and epithelial cell injury are most pronounced. Immunological changes and airway responsiveness to inhaled house dust mite (HDM) allergen and cholinergic antagonist will be evaluated periodically during the exposure regimen. Airway structure and pathology will be periodically monitored using contrast CT scans of the lungs. At completion of the protocol monkeys will be euthanized and the lung and airway tissue will be evaluated by a multidisciplinary group of investigators. This multidisciplinary approach ensures that the information from a given study is maximized.

These experiments are important to further our understanding of how environmental air pollutants effect lung development and exacerbate allergic asthma in developing animals and are especially timely given the increased incidence of allergic asthma in young children living in polluted urban environments.

**b) Procedures employed in this project:**

Please check the appropriate boxes if any of these procedures will be employed in your project:

- |   |   |   |
|---|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production **                  | <input type="checkbox"/> Food or water restriction        | <input type="checkbox"/> Special diets; food or water treatment.              |
| <input type="checkbox"/> Polyclonal Antibody Production **                  | <input type="checkbox"/> Non-recovery surgical procedures | <input checked="" 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 checked="" 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 checked="" type="checkbox"/> Fasting prior to a procedure.           | <input type="checkbox"/> Aversive conditioning.           | <input type="checkbox"/>  |

\*\* If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

1) Neonatal Monkeys: Under the direction of the primate center veterinarian staff experienced CRPRC personnel will separate neonatal monkeys of either sex from their mothers within the first three days of birth and reared under standard CRPRC nursery conditions. Appropriate housing conditions for infant monkeys will be established and maintained in the inhalation exposure chambers by the CRPRC staff. Infant monkeys will begin the protocol for ozone and/or allergen exposure (see below) when they reach 21-32 days of age.

2) Allergen Sensitization and Aerosol Exposure Protocol: Neonatal monkeys will receive one priming injection of HDM antigen between days 3 and 10 after birth. Monkeys will be primed once with an injection of allergen *Dermatophyoides farinae*, house dust mite extract, precipitated in aluminum hydroxide on day 0 (12.5 micrograms HDM extract precipitated in 10 mg alum in 1 ml given subcutaneously (SQ) on the back of the animal and  $2.5 \times 10^{11}$  killed *Bordetella pertussis* cells in 0.25 ml by the intramuscular route (IM). Further sensitizations will consist of aerosol of HDM allergen dissolved in phosphate buffered saline solution. The aerosol will be administered within the exposure chamber on days 3, 4, and 5 of each episodic ozone exposure. The exposure period will be 2 - 3 hours. Observation of the animals by an attending veterinarian and/or exposure personnel would be continuous during exposure. The concentration of HDM allergen to be aerosolized will be determined in conjunction with Mr. Tarkington, so as to approximate an inhaled total dose of 100 micrograms of allergen during each allergen exposure session. A small serum sample (3 ml/kg of body weight of whole blood) will be obtained from awake animals, with blood being drawn from a femoral vein, weekly for CBC and antibody analysis. On day 40 monkeys will be skin tested as described below for the selection process. If monkeys are not skin test positive on day 40, each will receive a second injection of HDM antigen in alum SQ. On days 68 and 82 skin testing will be repeated.

3) Skin Testing Protocol: Prior to the beginning of the antigen and/or ozone inhalation exposures all monkeys will be skin tested to determine the effectiveness of the sensitization protocol. Monkeys will be sedated using Ketamine hydrochloride (5-10 mg/kg, IM). Under anesthesia each monkey will be shaved on the lateral thorax. Three intradermal injections consisting of 0.1 ml of HDM antigen, histamine HCl, and phosphate buffered saline (diluent) will be made into the shaved area. The injection sites will be observed for wheal development for 30 minutes. At 20 minutes post-injection each site will be measured and the measurement will be recorded. Monkeys will be monitored for any adverse responses over the next 2 hours as they recover from anesthesia. In addition, blood (3 ml) will be obtained from each monkey from a site to be determined by the attending veterinarian immediately after induction of anesthesia and prior to skin testing and used as sample for HDM specific IgE and IgG ELISA as well as on total IgE ELISA.

4) Ozone exposure and animal housing. Monkeys will be housed during the study at the air pollution exposure facility at the CRPRC in specially designed inhalation exposure chambers suitable for long-term housing. This caging meets or exceeds space requirements in the ILAR Guide. On the days of ozone exposure (see attached protocol timeline) monkeys will inhale 0.2-1.0 ppm ozone for 4-8 hours. At all other times when in these chambers the monkeys will be breathing filtered air. For HDM allergen exposure the monkeys will be moved from these chambers and placed in identical cages within a different chamber for exposure as described in section 3 above.

5) Evaluation of Static Lung Mechanics and Aerosol Challenges for Evaluation of Airways Responsiveness via Transfer Impedance: Lung mechanics and airway hyperresponsiveness challenges will be performed at multiple time points during the experimental protocol. Monkeys will be sedated using Ketamine hydrochloride (5-10 mg/kg, IM). The monkeys will be anesthetized using Diprivan (0.1-0.2 mg/kg/min, IV) with the dose adjusted as deemed necessary by the attending veterinarian. Monkeys will be intubated with an appropriate sized cuffed endotracheal tube (2.5-3.5 mm). The monkey will first be placed in a whole body plethysmograph (Buxco Electronics Inc) and the intubation tube attached to a pneumatic valve.

To determine static lung mechanics and quasi-static lung compliance the monkey will spontaneously breathe through the valve to establish breathing frequency and tidal volume. Supplemental O<sub>2</sub> will be bled into the valve in order to maintain adequate arterial O<sub>2</sub> saturation levels. The monkey's lungs will then be inflated to 30cmH<sub>2</sub>O and allowed to deflate to FRC (functional residual capacity) to measure total lung capacity. The lungs will then be re-inflated to 30 cmH<sub>2</sub>O then rapidly deflated to negative 10 cmH<sub>2</sub>O to determine residual volume. Quasi-static lung compliance will be determined by allowing the monkey to spontaneously breathe against a

closed valve and both mouth pressure and box pressure will be measured in order to calculate quasi-static lung compliance.

After the static lung mechanics have been evaluated, the monkey will be moved to a head-out body plethysmograph (body box) and the intubation tube attached to a pneumatic four-way valve/pneumotachograph assembly. All challenges will be administered as aerosols at a set inflation pressure and breathing frequency (10.0 cmH<sub>2</sub>O and 30.0 bpm) using a compressed air nebulizer (Vortran, Inc., Miniheart Model) in series with a positive pressure ventilator (Bird Mark 7A respirator). The first aerosol challenge will be saline followed by doubling concentrations of histamine, phenyl biguanide (PBG) or methacholine with the initial concentration being 0.0625 mg/ml and ending at 64.0 mg/ml. The final concentration of aerosol to be delivered will be the concentration that doubles Raw (EC200Raw) or causes arterial O<sub>2</sub> saturation to fall below 75%. Allergen challenge will be done using a set concentration of house dust mite allergen (0.02 mg protein/ml) delivered at repeated five minute intervals separated by 60 second data collection periods. Allergen challenge will be terminated when airway resistance (Raw) doubles or arterial O<sub>2</sub> saturation falls below 75%. Data will be expressed as the cumulative dose of allergen (mass concentration of allergen in mg protein/ml x tidal volume in ml x number of breaths) that doubles Raw (CDA200Raw). The CDA200Raw and EC200Raw will be determined by linear interpolation on the log-log plot of the dose response curve with the response being expressed as the percent of baseline Raw.

For the evaluation of responsiveness (see above) pulmonary mechanics will be measured using a transfer impedance method. Briefly, the monkey breathes spontaneously through the pneumotachograph (Fleisch no. 2) while the thorax of the monkey is vibrated using a pseudo-random noise waveform encompassing frequencies of 2 to 128 Hz by two speakers mounted in the walls of the head-out plethysmograph (Pulmetrics Group, Boston, MA). The small changes in flow produced at the mouth are measured along with the changes in pressure inside the plethysmograph using a Microswitch transducer (model 743PC). This technique allows the monkey to breathe spontaneously while making pulmonary mechanics measurements at 4 second intervals. Concurrently, changes in lung compliance will be assessed by placing either a fluid or air-filled balloon catheter in the esophagus at the level of the heart to measure transpulmonary pressure. Pressure/flow signals will be collected and processed using a digital data acquisition system (PO-NE-MAH, Gould Instruments Inc) and lung compliance calculated for each dose of either allergen or histamine/PBG/methacholine.

7) Breathing Pattern and Arterial Oxygen Saturation. During the evaluation of airways responsiveness, tidal volume (VT) and breathing frequency will be recorded on a breath-by-breath basis by integrating the output of the pneumotachograph using a digital data acquisition system (PO-NE-MAH, Gould Instruments Inc). Arterial oxygen saturation (O<sub>2</sub>Sat%) will be recorded at the beginning and ending of each data collection period using a pulse oximeter.

8) Computed Tomography Scan. Animals will be transferred to the Radiology department at the VMTH for the procedure. The animals will be sedated with a cocktail of Ketamine (5-10mg/kg) and Medetomidine (30-50ug/kg) IM. Animals will be intubated using CRPRC SOP for intubation. The monkeys will be placed into the CT scanner and then given contrast medium (Conray 400, 2ml/kg) for vascular contrast. Animals will spontaneously breathe until the time of the actual scan, then the lungs will be step-wise inflated to 5, 10, 15, and 20 cm H<sub>2</sub>O pressure, utilizing an Ambu bag (fitted with a pop-off valve set to the desired pressure), to insure a constant volume. The airway pressure will be maintained for 30-60 seconds while the scan is completed. The scan may be repeated several times to insure high quality images. Following the procedure, the Medetomidine will be reversed with an equivalent dose of Atipamazole, also given IM.

9) Administration of Bromodeoxyuridine (BrdU). In order to identify lung cells undergoing DNA synthesis and replication all monkeys are to receive a single pulse label of BrdU at a dose of 50 mg/kg i.p. one hour before sacrifice. Animals are anesthetized with Ketamine (10mg/kg, IM), then the BrdU given via IP injection by a CRPRC animal health technician. BrdU is a detectable DNA nucleotide analog that is incorporated into the DNA of dividing cells. The BrdU will be prepared by the investigator and delivered to the animal health technicians in pre-loaded syringes. No special handling procedures are necessary other than wearing gloves and the used syringes should be disposed of just like other biohazardous waste.

**d) Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require**. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs (see attached Table for specific exposure protocols)	Number of Animals	Category
1	HDMA Sensitized/NOT exposed to HDMA Aerosol or Ozone.	12	1
2	HDMA Sensitized/exposed to ozone, but NOT exposed to HDMA Aerosol.	12	2
3	HDMA Sensitized/exposed to HDMA Aerosol, but NOT exposed to ozone.	12	2
4	HDMA Sensitized/exposed to HDMA Aerosol and ozone.	12	2
5	Monkeys to evaluate specific methods and protocols on young monkeys	8	1-2

#### Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p><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.</p>
2	<p>Minor stress or pain of short duration</p> <p><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</p>
3	<p>Moderate to severe distress</p> <p><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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p><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.</p>

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?

Though several other animal models of asthma have been utilized, including guinea pigs, rats and mice, they are all limited in that each one of these species differ greatly from humans in their immunology, airway neural control and airway morphology. We have chosen the rhesus monkeys because the structural and cellular aspects of their airway is very similar to that in humans and because the results of our previous studies that rhesus monkeys sensitized to HDM antigen represents a relevant model for human asthma with respect to antigen challenge and airway responsiveness.

Like humans rhesus monkeys show a wide range of responsiveness to HDM antigen sensitization and challenge. For this reason we have chosen a number of twelve animals per group in our first study to be conducted over the three years of the study. This number of animals was approximated using power analysis in which the mean baseline responsiveness to methylcholine of monkeys study in our pilot work along with the calculated standard deviation and assuming a desired responsiveness shift of one order of magnitude was used. We are also including a request for 8 additional monkeys (group 5). These additional 8 monkeys will be used to evaluate specific methods and protocols on young monkeys that have we have previously used in adults monkeys. This additional request was recommended by the CRPRC veterinary staff.

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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?
Rhesus Monkey	Ketamine HCl	10	IM/IV	Maintenance of sedation
Rhesus Monkey	Diazepam	0.1-1.0	IM/IV	Maintenance of sedation & muscle relaxation if needed.
Rhesus Monkey	Medetomidine	0.02-0.05	IM	Once, if deemed needed by attending veterinarian
Rhesus Monkey	Fentanyl	0.002-0.01	IM	Once, if deemed needed by attending vet.
Rhesus Monkey	Diprivan	0.1-0.2 mg/kg/min	IV	Adjusted as deemed necessary by attending vet
Rhesus Monkey	Atipamazole	30-50ug/kg	IM	Adjusted as deemed necessary by attending vet

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)

Ozone exposure may cause transient respiratory discomfort including substernal pain and cough. Repeated HDMA aerosol inhalation in HDMA sensitized animals may result in respiratory symptoms of bronchoconstriction, wheezing and cough.

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.

Discomfort of ozone inhalation is transient lasting 2-4 hours. If monkeys develop severe airway obstruction due to exposure to HDM allergen the attending veterinarian will deliver an aerosol of the bronchodilator albuterol to the monkey.

*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.*

**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?

2/1/02

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Current Contents	1989 to present	air pollution, asthma, lung
Medline	1980 to present	air pollution, asthma, lung

What were your findings with respect to alternative methodologies?

Though several other animal models of asthma have been utilized, including guinea pigs, rats and mice, they are all limited in that each one of these species differ greatly from humans in their immunology, airway neural control and airway morphology. Whole animal models are required to study asthma because of the multiple cell populations that are involved, including inflammatory and immune cells, epithelial cells, airway smooth muscle and airway sensory and efferent nerves.

Has this study been previously conducted?

Yes  No

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

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

After defined exposure and post-exposure periods, all animals will be euthanized for detailed study of the respiratory tract.

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
Monkeys	Overdose	Sodium pentobarbital	60 mg/kg	IV

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

Return them to nursery and colony.



