Comparative Histology of the Respiratory Tract of Normal Peromyscus Floridanus and P. Gossypinus and Effects of Exposure to Solid Rocket Motor Fuel Exhaust on P. Gossypinus

1977

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COMPARATIVE HISTOLOGY OF THE RESPIRATORY TRACT OF NORMAL PEROMYSCUS FLORIDANUS AND P. GOSYPINUS AND EFFECTS OF EXPOSURE TO SOLID ROCKET MOTOR FUEL EXHAUST ON P. GOSYPINUS

BY

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E.A., Southwestern At Memphis, 1969

THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Graduate Studies Program of the College of Natural Sciences of Florida Technological University

Orlando, Florida
1977
ABSTRACT

Microscopic examination of the tracheal dimensions of normal Florida mice (*Peromyscus floridanus*) and cotton mice (*P. gossypinus*) showed no significant differences between the two species, but external examination showed the tracheal length of the Florida mouse to be longer than that of the cotton mouse. Microscopic examination of the intrapulmonary apparatus (bronchioles, alveolar ducts, atria, and alveoli) of normal Florida and cotton mice showed no significant differences in measurements between the two species.

Cotton mice were exposed to exhaust gases produced by the burning of solid rocket motor (SRM) fuel. Mice exposed once for a duration of 10 min demonstrated an LD$_{50}$ of 52 to 56 ppm HCl/g body weight and an LD$_{50}$ of 169 to 173 mg Al$_2$O$_3$/m$^3$/g body weight. These LD$_{50}$ values suggested that SRM exhaust components may have a synergistic lethal effect when compared to the effects of individual components of the exhaust. Cotton mice exposed to the exhaust exhibited external signs of respiratory distress and dyspnea. Those mice that received lethal exposures showed internal signs of early inflammatory reactions. However, the most likely cause of death was a sudden shift in blood pH.
ACKNOWLEDGMENT

During the period of this study, many people provided assistance; to them, I express my appreciation.

I am especially indebted to my two graduate committee chairmen, Drs. John C. Mickus and I. Jack Stout; without them, the completion of my work would have been impossible. Dr. Mickus provided early guidance in the study and contributed considerably in the editing, even after his departure from the university. Dr. Stout provided patience, support, and many suggestions. I thank my committee members, Drs. Michael J. Sweeney and David W. Washington, for their suggestions during the study and criticisms of this thesis. Thanks are also due to Dr. James H. Price for advice in photomicrographic technique.

I thank Mr. and Mrs. Gary Byerley for their help in collecting and caring for the animal colony. My sister, Mrs. Martha Babb, helped in typing the manuscript.

My wife, Nancy, and my mother deserve special acknowledgment for their support and encouragement. Nancy also helped with the typing.

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INTRODUCTION

Within the next few years the Kennedy Space Center will become the prime launching and landing site for the space shuttle program. Exhaust products from the solid rocket motors (SRM) will enter the atmosphere of the Kennedy Space Center (KSC) and the surrounding area. Effects of exhaust products on selected components of the ecosystems have been studied (Nimmo et al., 1974a; Nimmo et al., 1974b; DeGuehery, 1976; Stout et al., 1976).

Merritt Island National Wildlife Refuge has a large variety of animal life (Ehrhart, 1976) and it is desirable to determine the possible effects of the SRM exhaust on animals indigenous to the area. The cotton mouse (*Peromyscus gossypinus*), a native small mammal, was selected for study.

This study determined an LD$_{50}$ for cotton mice exposed to various concentrations of SRM exhaust. The histological effect of the exhaust on the respiratory tract was determined and a cause of death was hypothesized. Since no literature concerning the respiratory tract of the animal was available, control studies of cell types and structural dimensions of the respiratory tree were undertaken. Another native mammal, the Florida mouse (*Peromyscus floridanus*), was studied for comparison to the cotton mouse.
LITERATURE REVIEW

Solid Rocket Motor Fuel Exhaust Products

A review of the literature reveals that no experimentation has been undertaken in which mammals have been directly exposed to SRM exhaust. Information does exist, however, on the effects of various components of the exhaust on organisms. The nine major theoretical combustion products which account for 99 percent of the original fuel are listed in Table 1. Actual launch data showed that significant quantities of FeCl₂ were produced, thus the actual exhaust cloud contains elements other than those present in the fuel (Vickers, 1974). Cesta and McLouth (1969) proposed that the actual exhaust cloud mixes rapidly with the air, thus making the determination of its composition somewhat impractical.

The theoretical combustion products most likely to exert harmful effects are CO, HCl, Al₂O₃, and Cl gas. Experimental results have shown that there is an afterburning of the CO to CO₂ (Rhein, 1973). Due to its low mole fraction, the effects of Cl gas should be minimal. HCl and Al₂O₃ are the remaining combustion products which are of concern.

HCl is a colorless, hygroscopic gas. Its high solubility in water makes it easily transformed into a mist or aerosol. At 25°C and 760 mm of mercury, 1.49 mg of HCl/m³ of air is equivalent to 1 ppm (Rowe et al., 1971). Cesta and McLouth (1969) studied the concentration of HCl in SRM exhaust during several Titan III-C
Table 1. Major theoretical combustion products of solid rocket motor fuel (Vickers, 1974).

<table>
<thead>
<tr>
<th>Product</th>
<th>Mole Fraction</th>
</tr>
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<tbody>
<tr>
<td>$\text{H}_2$ gas</td>
<td>0.32008</td>
</tr>
<tr>
<td>CO gas</td>
<td>0.25761</td>
</tr>
<tr>
<td>HCl gas</td>
<td>0.14744</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$ gas</td>
<td>0.09663</td>
</tr>
<tr>
<td>N$_2$ gas</td>
<td>0.07801</td>
</tr>
<tr>
<td>Al$_2$O$_3$ solid</td>
<td>0.07778</td>
</tr>
<tr>
<td>CO$_2$ gas</td>
<td>0.01407</td>
</tr>
<tr>
<td>H gas</td>
<td>0.00506</td>
</tr>
<tr>
<td>Cl gas</td>
<td>0.00170</td>
</tr>
</tbody>
</table>
launches. A summary of their measurements of HCl concentrations at ground level (15.24 to 45.72 m) is presented in Table 2. The theoretical HCl concentration in their study was estimated to be 810 ppm after 13 sec. Cesta et al. (1967) estimated the HCl concentration to be essentially 0 ppm at 45 sec after ignition. Cicerone et al. (1973) estimated the HCl concentration to be 301 ppm at $T = 90$ sec and 500 m altitude. The majority of the exhaust clouds studied rose vertically until cloud temperatures approximated that of outside air, usually at a height of 152.4 to 304.8 m (Cesta et al., 1967). Another source (NASA anon undated) estimated the ground cloud to rise to about 792.5 m. Most ground clouds drift downwind and disperse without returning to ground level, thus presenting no toxic hazards (Cesta and McLouth, 1969). The ground cloud is predicted to eventually return to ground level with HCl concentration of 3.7 ppm at the extreme (NASA anon undated).

The maximum ground cloud HCl concentration predicted for the space shuttle boosters is only 60 percent greater than Titan III-C boosters when calculated with the same parameters (NASA anon undated). The space shuttle should release five times more HCl but this would be offset by the greater bouyancy of the ground cloud which would go to higher altitudes.

Cicerone et al. (1972) summarized the work of several researchers on the effects of various concentrations of HCl on laboratory animals over different time periods. The animals used were rabbits, cats, guinea pigs, pigeons, and monkeys. Lower concentrations (below 1,500 ppm) maintained over a long time have
Table 2. Summary of HCl concentrations at ground level (15.24 to 45.72 m) during several Titan III-C launches (Cesta and McLouth, 1969).

<table>
<thead>
<tr>
<th>Sample time</th>
<th>Range (ppm HCl)</th>
<th>Average (ppm HCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T +4 to T +14</td>
<td>1340-5900</td>
<td>3246</td>
</tr>
<tr>
<td>T +14 to T +44</td>
<td>0-535</td>
<td>253</td>
</tr>
<tr>
<td>T +44 to T +120</td>
<td>0-9</td>
<td>3</td>
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</table>

T is seconds after ignition.
very similar effects to a high concentration (3,400 to 4,300 ppm) over a short time. Flury and Zernick (1931) and Machle et al. (1942) have also studied effects of various concentrations and exposure lengths of HCl on cats, rabbits, guinea pigs, and monkeys. Stahl (1969) has reviewed much of the literature on HCl exposure. Results from these studies have related only gross anatomical changes and no histological data were provided.

Safe exposure limits to HCl for man have been determined. Rowe et al. (1971) described the Public Emergency Limits and the Short-Term Public Limits to which humans may be exposed with no expected health hazard as 7 and 4 ppm, respectively.

The concentration of Al₂O₃ in the SRM exhaust has been estimated to be about 318 particles/cm³ at an altitude of 500 m (Cicerone et al., 1972). The return of the ground cloud to earth yields an Al₂O₃ concentration of four 10 μ particles/cm³ which is similar to dusty air (NASA anon undated). About 94.2 percent of the Al₂O₃ in the SRM exhaust has a particle size less than 0.1 μ (Nadler, 1973). The Al₂O₃ is almost entirely γ-Al₂O₃ with about 1 percent α-Al₂O₃.

Gardner and Cummings (1933) intravenously injected rabbits with Al₂O₃ particles 1 to 3 μ in diameter. The particles were phagocytosed and produced no fibrosis. Clark (1956) found the effects of Al₂O₃ on mice to be typical of bronchopneumonia with congestion of blood vessels, much infiltration by leukocytes and lymphocytes, edema, and consolidation in some areas. Stacy et al. (1959) found γ-Al₂O₃ to be quite fibrogenic.
Palm et al. (1956) studied the effects of anatomical and physiological differences among small animals on the retention of particulate matter. Smaller animals were found to retain smaller particulate matter within certain areas of the respiratory system and to have higher efficiency of upper respiratory tract retention than larger animals. Since smaller animals ventilate faster, the rate of deposition will be greater in their system. The above information exists even in the light of the fact that pulmonary air spaces are essentially the same in small animals and man (Hatch and Gross, 1964).

Cicerone et al. (1972) studied Shaver's disease in humans and concluded that the effects of the Al$_2$O$_3$ particles on the lung depend on a combination of particle size, length of exposure, and dosage rate. About 60 percent of the particles 2 $\mu$ or less were able to penetrate the alveolar regions and to be retained.

Particles greater than 10 $\mu$ in diameter are assumed to be retained in the nasal region (Hatch and Gross, 1964). The upper respiratory tract is very efficient for removing particles greater than 2 $\mu$ (Cicerone et al., 1973). Spencer (1968), however, found the optimal size for human pulmonary retention to be 2.5 $\mu$. Particles having a diameter of 0.5 $\mu$ have the minimum probability of respiratory deposition owing to the fact that the combined forces of diffusion and precipitation by sedimentation have a minimum value at this size (Hatch and Gross, 1964). The rate of deposition increases, however, above and below this size.

Respiratory systems of mammals are efficient in removing
particulate matter, provided no hindrance exists. The HCl could hinder the removal of Al$_2$O$_3$ particles. Also, the HCl and Al$_2$O$_3$ could be bound together, thus faster transport of Al$_2$O$_3$ to the alveoli could be obtained due to synergistic action. Cesta et al. (1967) found HCl in the SRM exhaust not to be reduced by chemical combination with Al$_2$O$_3$. Cicerone et al. (1973) stated that they planned in subsequent studies to determine if bonding between the two products occurs. Baldwin (1974) had no success in his attempt to determine if HCl is adsorbed onto Al$_2$O$_3$.

**Comparative Study of Respiratory Systems**

In order to evaluate the effects of SRM exhaust, it was necessary to document the normal histology of the respiratory tree of the cotton mouse. No information was discovered in the literature concerning the respiratory tree of any member of the genus *Peromyscus*. For comparative purposes, the normal histology of the respiratory tree of the Florida mouse was also studied.

The cotton mouse is indigenous to the southeastern United States, with the exception of mountainous regions (Colley, 1962). The head and body length averages 9.14 to 11.68 cm and body weight averages 28.0 to 51.0 g (Burt and Grossenheider, 1964). In contrast, the Florida mouse is indigenous to central and southeastern Florida (Hall and Kelson, 1959). The head and body length averages 11.18 to 12.70 cm (Burt and Grossenheider, 1964) and body weight averages 25.0 to 49.0 g (Layne, 1976). It was hypothesized that adaptation to a much larger and more diverse range could possibly have more generalized structural features within the respiratory
system of the cotton mouse; whereas the Florida mouse may exhibit special respiratory adaptations to avoid moisture loss.
MATERIALS AND METHODS

Animals

The animals were collected from natural habitats found on the campus of Florida Technological University, Orlando, Florida. The Florida mouse was collected in predominantly sand pine scrub areas while the cotton mouse was collected in pine flatwoods, sand pine scrub, and disturbed pine woods. Both species were trapped in Sherman live traps baited with oat flakes and peanut butter.

Captured animals were weighed and marked with numbered monel ear tags. Weights were recorded monthly thereafter. Animals were maintained in plastic cages (32 x 18 x 13 cm) which contained cedar chips and cotton for nesting. One male and two females, conspecific, were housed together to establish a breeding stock. Wayne Lab-Blox F6, 4 g per animal per day, supplemented weekly with a few sunflower seeds was provided. Water was supplied ad libitum. The animals were maintained in an air-conditioned trailer. Room temperature averaged 22°C and relative humidity varied between 45 to 65 percent. The photoperiod was 12:12 light:dark cycle.

Histology

Animals were euthanatized with an overdose of sodium pentobarbital injected intraperitoneally. Weights were recorded to the nearest 0.1 gram. The trachea, lungs, heart, and esophagus were immediately removed and rinsed in isotonic saline. Pulmonary blood was expressed by compressing the heart. The tissues were fixed in
10 percent buffered formalin for a minimum of one week. The esophagus and heart were dissected away and the respiratory structures were dehydrated by immersion in alcohol baths (30, 50, 70, 85, 95, and 100 percent), cleared in toluene, and embedded in Paraplast horizontally with the dorsal surface down. In a few instances the trachea was ligated slightly anterior to the carina and the right and left lobes were also separated at the carina. Each of these portions was embedded separately as horizontally as possible with the dorsal surface down. All tissue was sectioned longitudinally at 6 μm beginning at the dorsal surface and was stained with Harris hematoxylin and eosin Y (Humason, 1972). Cover slips were applied with Histoclad.

Measurements

External lengths and diameters of the preserved tracheas of adult cotton and Florida mice were measured to the nearest 0.4 mm with Helios calipers. Length was measured on the dorsal surface from the posterior edge of the cricoid cartilage to the carina. Diameter was measured at the approximate midpoint of the trachea. The internal lengths and diameter of the tracheas were measured from prepared slides using a Gillette and Siebert projection microscope. The reference points were the same as those used for the external measurements. The number of cartilage rings was also recorded.

Histological measurements were taken with an American Optical Model 150 light microscope at 100X to 1000X. The following tracheal parameters were measured with a Bausch and Lomb ocular micrometer: cilia height, epithelial cell height, and submucosa to cartilage
thickness. Goblet cells, seromucous glands, and mononuclear cells were noted as being present, absent, or few in number. Error associated with measurements was ± 0.4 μm. The following parameters were measured in the lung bronchioles: proximal and distal lumen diameter, epithelial cell height at the terminal end, and smooth muscle layer thickness at the terminal end. Goblet cells and mononuclear cells were noted as being present, absent, or few in number. Close proximity to blood vessels was also noted. The following parameters were measured in the alveolar apparatus: alveolar duct length and diameter, atria diameter, and alveoli diameter. The number of branches of the alveolar duct and the presence of mononuclear cells were also noted.

Statistics

The mean, standard error of the mean, and coefficient of variation were calculated for each structure measured on the two species. A student t-test comparing the means was performed.

Photography

Photographs were taken on a Zeiss microscope mounted with a Zeiss camera. Kodax color photomicrographic film was used.

Exposure Chamber

An exposure chamber was designed by Dr. John C. Mickus and constructed with some modifications by the Bendix Corporation, Kennedy Space Center. The chamber was designed to fulfill the following requirements:

1. to separate the animals from the heat generated by fuel ignition,
2. to be small enough to accumulate high concentrations of exhaust,
3. to provide an even distribution of the test gases and particles,
4. to permit the monitoring of temperature and humidity,
5. to permit the monitoring of gas and particle concentration,
6. to be easily cleaned, and
7. to be safe to operate.

The 38 liter chamber consisted of two identical domed compartments constructed of 0.64 cm wall acrylic tubing with a 25.4 cm outside diameter and a height of 20.32 cm. The top and bottom areas of each compartment were fabricated by forming sheets of 0.96 cm acrylic into domes contoured to the 25.4 cm outside diameter of the compartments. The compartments were connected by two curved acrylic tubes with 0.64 cm walls and an outside diameter of 5.08 cm. One curved tube joined the two domed compartments at their tops, the other curved tube joined them at their bottoms. All portions of the chamber were bonded and sealed with the acrylic bonding compound "Plexite". The left compartment was designated as the burn compartment and the right side as the specimen compartment (Fig.1). Transparent acrylic tubing was used because it is non-reactive with acids and would not introduce chemical by-products into the closed loop system of the test chamber.

Openings of 14.37 cm by 17.78 cm were cut into each compartment to allow installation and access to the fixed components. A hinged acrylic door with a rubber gasket covered these openings during testing. The fixed components of the burn compartment consisted of an igniter, a burn pan with a mesh screen cover, a circulation fan, and ports for temperature and humidity probes (Fig. 2). The access
Fig. 1. Exposure chamber used to expose cotton mice to solid rocket motor exhaust. A, burn compartment; B, specimen compartment; C, control panel; D, sampling system.
Fig. 2. Components of the exposure chamber burn compartment. A, igniter; B, burn pan without screen cover; C, circulation fan.
door to the specimen compartment contained two air locks to allow movement of specimens into and out of the compartment. Each air lock was a 40.64 cm acrylic tube with a 7.62 cm inside diameter. Both air lock tubes were bonded onto the door with "Plexite". Specimen cages were designed to fit inside the air lock tubes and to hang on the air lock support frames when pushed into the specimen compartment (Fig. 3).

The air lock system provided an area where the specimens could be loaded into the cages and held in the tubes while breathing normal air. When the cages were pushed entirely into the air locks, the total specimen area was sealed by O-rings from both outside air and the exhaust cloud inside the chamber. When desirable the test animals were injected into the specimen compartment thus maintaining the sealed system. A drain cock was provided in the bottom connecting tube.

The chamber was mounted on a metal base. The control panel consisted of a primary power circuit breaker switch, a power indicator light, a push button control of ignition (with a separate safety switch), and a speed control and voltage meter for the fan. Power for the fan was provided by a 17 VDC power supply. Separate circuit breakers protected the system power. The panel unit also provided 110 VAC for external use. The chamber was operated under a hood to facilitate venting of the exhaust cloud after each exposure.

The system provided a means whereby up to 6.5 g of fuel could be safely burned, while generating an exhaust cloud of sufficient
Fig. 3. Specimen compartment of the exposure chamber.
toxicity to elicit acute effects.

Exposure Protocol

The following steps were followed for each animal exposure:

1. A block of solid rocket fuel, provided by the Jet Propulsion Laboratory, Pasadena, California, was placed in the burn pan and attached to the igniter filament. The wire frame was placed over the pan and the burn compartment door closed and sealed.

2. Five (four on some occasions) cotton mice of the same sex and similar weight were placed in the cages, three in the bottom cage and two in the top cage, and the cages locked onto their air lock support frames. The cages were pushed inward and stopped in a position so that normal air was available for respiration.

3. The primary power circuit breaker was turned on, causing the power indicator light to turn on.

4. The fan was turned on and adjusted to the required speed by observing the fan voltage meter. The fan was normally run at 5.0 volts and turned off 0.5 minutes after the ignition.

5. The exhaust hood which covered the chamber was turned on.

6. The safety switch cover guard was raised and the switch turned to the ON position in order to arm the igniter button.

7. The fuel was ignited by pushing in and holding the igniter push button until the start of the burn. The release of the button cut all power to the igniter.

8. When the temperature in the specimen compartment dropped to 30°C and the exhaust cloud had equilibrated in both compartments, the air lock cage support frames were injected into the compartment to permit exposure to the fumes.

9. Temperature and flow meter readings in the compartment were taken at approximately two minute intervals during the 10 minute exposure period. The relative humidity of the compartment was infrequently determined with a humidity sensor made by Panimetrics. Behavior patterns were noted when the cloud permitted observation.

10. The system's power was cut off by turning off the primary circuit breaker.

11. Animals were removed and the following conditions observed: respiratory pattern, saliva in mouth, nose irritation, and fur damage. Dead animals were immediately processed using the same procedure employed with normal animals. Gross abnormalities were noted. Living animals were placed in their cages.
Animals which died within 24 hours were considered to be exposure mortalities.

12. The chamber was vented, washed with water, and dried. When large concentrations were desired the chamber walls were not cleaned, thus more of the exhaust cloud stayed in suspension rather than adsorbing onto the walls.

Analysis of SRM Exhaust

Analysis of the SRM exhaust was done by Dr. Brooks C. Madsen, Florida Technological University (Madsen, 1974a; Madsen, 1974b). A continuous sample of the exhaust cloud was drawn from the specimen compartment through a midget impinger connected in series with a glass-fitted dispersion tube. Both devices were immersed in deionized water. SRM exhaust was withdrawn at approximately 0.2 liters per minute with a rotameter-type flow meter and vacuum pump. The components of the sampling system were connected by 0.96 cm outside diameter tygon hose with glass connections (Fig. 4).

Hydrogen Chloride Analysis

After sampling, the impinger and dispersion tube's contents were rinsed with deionized water and all of the solutions combined. The concentration of hydrogen chloride (HCl) was determined by measuring the concentrations of chloride ions. One ml of 2.00 M sodium nitrate was added for every 20 ml of the sampling solution in order to adjust the ionic strength. The chloride ion concentration was determined potentiometrically using silver-silver chloride indicator electrodes. The reference electrode was in electrical contact with external solutions through a 10 percent potassium nitrate, 3 percent agar salt bridge. The internal electrode was determined with a Beckman Model 76 Expandomatic pH
Fig. 4. Components of the exposure chamber sampling system. A, impinger; B, dispersion tube; C, rotameter-type flow meter; D, vacuum pump.
meter in the expanded range. The molar concentration of chloride was found from a calibration curve determined from the potential differences between standard chloride solutions. The time integrated average concentration of hydrogen chloride in the exposure chamber was calculated using the equation developed by Madsen (1974a).

**Aluminum Oxide Analysis**

The concentration of aluminum oxide (Al₂O₃) in the SRM exhaust was determined by measuring the concentration of alumina. After hydrogen chloride analysis was completed, the remaining solution was tested by turbidimetry for alumina. A Bausch and Lomb Spectronic 20 spectrophotometer operated at 400 nm using 1.17 cm pathlength cuvettes was utilized. The concentration of alumina was determined from a calibration curve generated from samples recovered from the scrubber system. The concentration of aluminum oxide was calculated from the equation developed by Madsen (1974b).

**Pathology**

Dissection of cotton mice that died during exposure was performed immediately after their removal from the exposure chamber. Gross abnormalities were observed. The trachea and lungs were prepared as previously described for normal specimens. Pathological consultation was provided by Dawson Research Corporation, Orlando, Florida.

**Exposure Statistics**

The regression line for concentration of both hydrogen chloride and aluminum oxide and percent mortality was determined. \( \text{LD}_{50} \)
determinations for both substances were calculated from the regression lines. A test for correlation of percent mortality and concentration of hydrogen chloride and aluminum oxide was performed.
RESULTS

The dimensions of the various parts of the respiratory tree are reported in two parts: (1) the extrapulmonary apparatus (the trachea), and (2) the intrapulmonary apparatus (the bronchioles, alveolar ducts, atria, and alveoli). All measurements apply to normal animals.

The Extrapulmonary Apparatus

Data on external measurements of preserved specimens are summarized in Table 3. Histological data obtained from study of longitudinal sections of the trachea of the two species are summarized in Table 4. Each species is described separately in the following sections.

Florida Mouse

Tracheal length measured by calipers averaged 6.75 mm (Table 3); whereas, mean length measured on longitudinal sections was 5.75 mm (Table 4). The external diameter averaged 2.08 mm and the lumina 1.04 mm. Most specimens had 17 cartilage rings, but in a few instances 18 or 19 rings were noted. The epithelial layer consisted of pseudostratified ciliated columnar cells which rested on a thin basement membrane (Fig. 5). Mean height of the columnar cells was 12.55 μm and the moderately abundant cilia measured 4.12 μm in length. Nuclei of the columnar cells were ovoid to elongated in shape and were 1/3 to 1/2 the size of the cell. A few non-ciliated columnar cells were noted. The basement membrane contained basal
Table 3. Measurements of preserved tracheas of Florida mice and cotton mice.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Florida Mouse</th>
<th>Cotton Mouse</th>
<th>t_{diff}^*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>C.V.</td>
</tr>
<tr>
<td>Length, mm</td>
<td>18</td>
<td>6.75±0.13</td>
<td>8.03</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>18</td>
<td>2.08±0.05</td>
<td>9.63</td>
</tr>
</tbody>
</table>

Mean is ± Standard Error

C.V. is Coefficient of Variation

*= P<0.05

NS is not significant
Table 4. Observations made on microscopic examination of longitudinal sections of the trachea of Florida mice and cotton mice (N = 10 in each case).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Florida Mouse</th>
<th></th>
<th></th>
<th>Cotton Mouse</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observations</td>
<td>Mean</td>
<td>C.V.</td>
<td>Observations</td>
<td>Mean</td>
<td>C.V.</td>
<td>t&lt;sub&gt;diff&lt;/sub&gt;</td>
</tr>
<tr>
<td>Length, mm</td>
<td>42</td>
<td>5.75±0.24</td>
<td>13.24</td>
<td>74</td>
<td>5.27±0.17</td>
<td>10.09</td>
<td>-0.18 NS</td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>40</td>
<td>1.04±0.06</td>
<td>18.03</td>
<td>75</td>
<td>0.85±0.06</td>
<td>20.93</td>
<td>-0.42 NS</td>
</tr>
<tr>
<td>Cartilage rings</td>
<td></td>
<td>17</td>
<td></td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cilia height, μm</td>
<td>123</td>
<td>4.12±0.11</td>
<td>8.70</td>
<td>231</td>
<td>4.31±0.13</td>
<td>9.74</td>
<td>0.10 NS</td>
</tr>
<tr>
<td>Columnar cell height, μm</td>
<td>120</td>
<td>12.55±0.56</td>
<td>14.04</td>
<td>210</td>
<td>17.83±1.09</td>
<td>19.40</td>
<td>0.74 NS</td>
</tr>
<tr>
<td>Basement membrane to cartilage width, μm</td>
<td>118</td>
<td>20.27±2.00</td>
<td>31.25</td>
<td>141</td>
<td>31.08±2.24</td>
<td>22.75</td>
<td>0.89 NS</td>
</tr>
<tr>
<td>Goblet cells</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seromucous glands</td>
<td>++</td>
<td></td>
<td></td>
<td>++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Observations represent the total number of measurements made in all of the 10 specimens for each species.

-, Absent; +, Few in number; ++, Numerous

Mean is ± Standard Error

C.V. is Coefficient of Variation

* = P < 0.05
Abbreviations: A, alveoli; AD, alveolar duct; AS, alveolar sac; BC, basal cells; CA, cartilage; CC, columnar cell; CI, cilia; CU, cuboidal cell; LC, low columnar cell; SG, seromucous gland.

Fig. 5. Longitudinal section through the trachea of a Florida mouse (1,000 X).

Fig. 6. Longitudinal section through the trachea of a Florida mouse (160 X).

Fig. 7. Longitudinal section through the lung of a Florida mouse showing terminal bronchiole (160 X).

Fig. 8. Longitudinal section through the lung of a Florida mouse (100 X).
cells which were ovoid to round and not always distinct. Goblet cells appeared to be absent; however, gaps did exist between some columnar cells.

The submucosal region, 20.27 μm in thickness, contained numerous seromucous glands located mostly between cartilage rings (Fig. 6). These glands were concentrated more in the anterior portion of the trachea. Mononuclear cells appeared infrequently along with some granulocytes in the fibroconnective tissue of the submucosa.

Cotton Mouse

Tracheal length measured by calipers averaged 6.18 mm (Table 3). The mean length on longitudinal sections was 5.27 mm (Table 4). The external diameter averaged 2.10 mm and the lumina 0.85 mm. The number of cartilage rings was usually 17. Pseudostratified ciliated columnar cells of the epithelium were 17.83 μm in height with ovoid to elongated nuclei. Nuclei were 1/3 to 1/2 of the cell's height. The moderately abundant cilia measured 4.31 μm in length. A few non-ciliated cells were noted. The basement membrane contained ovoid to round basal cells which were not always distinct. Goblet cells were absent from the epithelial layer; however, a few gaps did exist between some columnar cells.

The submucosal region was 31.08 μm thick and contained numerous seromucous glands located mostly between cartilage rings toward the anterior end. Mononuclear cells appeared infrequently along with some granulocytes in the fibroconnective tissue of the submucosa.

The Intrapulmonary Apparatus

The lungs of both the Florida mouse and cotton mouse appeared as
described for lab mice by Innes et al. (1967) and Hummel et al. (1966). The right lung consisted of four lobes (apical, cardiac, azygos, and diaphragmatic). The entire right lung was larger in anatomical mass than the single left lobe. At death the lungs of both species were a light pink color, soft and crepitant with no spots of any kind (Innes et al., 1967).

A summary comparison of the data collected by microscopic examination of the bronchioles is presented in Table 5. Data concerning alveolar ducts, atria, and alveoli are presented in Table 6.

**Florida Mouse**

The diameter of the lumen of the bronchioles varied from a mean of 189.65 μm at the proximal end to 57.20 μm at the distal end. Epithelium of the terminal bronchioles consisted of low columnar and cuboidal cells (Fig. 7). Mean height of the columnar cells was 11.48 μm and the mean for the cuboidal cells was 7.84 μm. Cilia were absent in the distal portion of the bronchioles and quite difficult to observe in the proximal end. The smooth muscle layer at the distal end had an average thickness of 6.66 μm. Goblet cells were absent but a few mononuclear cells were present. Large blood vessels were located adjacent to the bronchioles.

The mean length of the alveolar ducts was 188.06 μm and the mean diameter was 43.55 μm. Epithelium of the walls was composed of squamous cells with a few cuboidal cells at places where the walls projected into the lumen (Fig. 8). As the alveolar ducts bifurcated each gave rise to two and sometimes three atrial sacs. The lumen of
Table 5. Observations made on microscopic examination of longitudinal sections of the bronchioles of Florida mice and cotton mice (N = 10 in each case).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Florida Mouse</th>
<th></th>
<th></th>
<th>Cotton Mouse</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observations</td>
<td>Mean</td>
<td>C.V.</td>
<td>Observations</td>
<td>Mean</td>
<td>C.V.</td>
</tr>
<tr>
<td>Lumen diameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>proximal, $\mu$m</td>
<td>250</td>
<td>189.65±4.81</td>
<td>8.03</td>
<td>255</td>
<td>158.43±4.94</td>
<td>9.86</td>
</tr>
<tr>
<td>distal, $\mu$m</td>
<td>250</td>
<td>57.20±1.91</td>
<td>10.54</td>
<td>251</td>
<td>55.21±3.98</td>
<td>22.79</td>
</tr>
<tr>
<td>Epithelial cell height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low columnar, $\mu$m</td>
<td>250</td>
<td>11.48±0.12</td>
<td>3.50</td>
<td>251</td>
<td>11.72±0.21</td>
<td>5.60</td>
</tr>
<tr>
<td>cuboidal, $\mu$m</td>
<td>250</td>
<td>7.84±0.09</td>
<td>3.58</td>
<td>231</td>
<td>8.24±0.18</td>
<td>6.77</td>
</tr>
<tr>
<td>Smooth muscle, $\mu$m</td>
<td>250</td>
<td>6.66±0.22</td>
<td>10.27</td>
<td>251</td>
<td>6.45±0.25</td>
<td>12.35</td>
</tr>
<tr>
<td>Goblet cells</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Observations represent the total number of measurements made in all of the 10 specimens for each species.

- Absent; +, Few in number; ++, Numerous

Mean is $\pm$ Standard Error

C.V. is Coefficient of Variation

* $= P<0.05$
Table 6. Observations made on microscopic examination of longitudinal sections of the alveolar apparatus of Florida mice and cotton mice (N = 10 in each case).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Florida Mouse</th>
<th>Cotton Mouse</th>
<th>t_{diff}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observations</td>
<td>Mean</td>
<td>C.V.</td>
</tr>
<tr>
<td>Alveolar duct</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>length, μm</td>
<td>250</td>
<td>188.06±1.70</td>
<td>2.86</td>
</tr>
<tr>
<td>diameter, μm</td>
<td>250</td>
<td>43.55±0.90</td>
<td>6.55</td>
</tr>
<tr>
<td>number of branches</td>
<td>2 (few 3's)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atria diameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wall to wall, μm</td>
<td>251</td>
<td>123.08±3.51</td>
<td>9.01</td>
</tr>
<tr>
<td>alveoli edges, μm</td>
<td>251</td>
<td>52.34±2.31</td>
<td>32.64</td>
</tr>
<tr>
<td>Alveoli diameter, μm</td>
<td>252</td>
<td>43.88±1.21</td>
<td>8.74</td>
</tr>
</tbody>
</table>

Observations represent the total number of measurements made in all of the 10 specimens for each species.

Mean is + Standard Error

C.V. is Coefficient of Variation

* = P<0.05
the atria was 52.43 μm and the entire internal diameter, including alveoli, was 123.08 μm. The atria were lined with squamous cells with a few cuboidal cells projecting into the lumen (Fig. 9). Likewise, alveoli were lined with squamous cells, varied in shape, and averaged 43.88 μm in diameter (Fig. 10).

**Cotton Mouse**

The lumen of the bronchioles varied from a mean diameter of 158.43 μm at the proximal end to 55.21 μm at the distal end. Epithelium of the terminal bronchioles consisted of low columnar and cuboidal cells with mean heights of 11.72 μm and 8.24 μm, respectively. Cilia were absent from the epithelium at the distal portion and occurred infrequently at the proximal end. The smooth layer had a mean thickness of 6.45 μm. No goblet cells were observed, but a small number of mononuclear cells was present. The bronchioles were lined by large blood vessels, usually on one side but sometimes on both sides.

The mean length of the alveolar ducts was 203.33 μm and the mean diameter was 42.47 μm. Squamous cells formed the epithelium of the walls with a few cuboidal cells at places where the walls projected into the lumen. Alveolar ducts bifurcated and each one gave rise to two and sometimes three atrial sacs. The atrial lumen were 52.37 μm and the entire internal diameter, including alveoli, averaged 114.37 μm. The atria were lined by squamous cells with a few cuboidal cells projecting into the lumen (Fig. 9). Alveoli were numerous and varied in shape. They were lined with squamous cells and had a mean diameter of 39.27 μm (Fig. 10).
Fig. 9. Longitudinal section through the lung of a cotton mouse (400 X).

Fig. 10. Longitudinal section through the lung of a cotton mouse (1,000 X).
Exposure Statistics

The mortality rates of cotton mice are compared to the weight of the fuel burned plus theoretical and actual experimental concentrations of HCl and Al$_2$O$_3$ in Table 7. Experimental concentrations were found to be much lower than the theoretical yields in all cases. Thus, experimental concentrations could not always be increased simply by increasing the weight of the fuel. An exposure of special significance involved the burning of 5.24 g of fuel which yielded a high HCl concentration. However, no deaths resulted. Owing to this condition, statistical data were computed using all of the exposures ($N = 11$) and without the exposure to 5.24 g ($N = 10$).

A test of the relationship between the experimental concentration of HCl and percent mortality indicated the two variables were correlated when all exposures were considered ($N = 11$, $r = 0.68$, and $p < 0.05$). The correlation increased when the one questionable exposure was eliminated ($N = 10$, $r = 0.76$, and $p < 0.05$). The same test was performed to study the correlation between the experimental concentration of Al$_2$O$_3$ and the percent mortality. The results indicated a positive correlation to exist when the questionable exposure was not used ($N = 10$, $r = 0.80$, and $p < 0.05$) but a lack of correlation when the extra exposure was used ($N = 11$, $r = 0.58$, and $p < 0.05$).

The regression lines for percent mortality and concentration of HCl and Al$_2$O$_3$ are presented in Figs. 11 and 12, respectively. Two regression lines are presented for both HCl and Al$_2$O$_3$ owing to the
Table 7. Mortality response of cotton mice exposed for 10 minutes to SRM exhaust.

<table>
<thead>
<tr>
<th>Fuel (g)</th>
<th>Mean Body Weight (g)</th>
<th>HCl Concentration (ppm)</th>
<th>Al₂O₃ Concentration (mg/m³)</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experimental</td>
<td>Theoretical</td>
<td></td>
</tr>
<tr>
<td>4.30</td>
<td>28.10</td>
<td>993</td>
<td>15,200</td>
<td>0/5</td>
</tr>
<tr>
<td>4.97</td>
<td>29.30</td>
<td>913</td>
<td>17,700</td>
<td>0/5</td>
</tr>
<tr>
<td>5.00</td>
<td>32.10</td>
<td>1,250</td>
<td>17,800</td>
<td>1/5</td>
</tr>
<tr>
<td>5.15</td>
<td>23.30</td>
<td>860</td>
<td>18,300</td>
<td>0/5</td>
</tr>
<tr>
<td>5.20</td>
<td>33.22</td>
<td>1,280</td>
<td>18,600</td>
<td>0/5</td>
</tr>
<tr>
<td>5.24</td>
<td>24.75</td>
<td>1,590</td>
<td>18,600</td>
<td>0/4</td>
</tr>
<tr>
<td>5.30</td>
<td>26.60</td>
<td>1,170</td>
<td>18,900</td>
<td>3/5</td>
</tr>
<tr>
<td>5.30</td>
<td>31.54</td>
<td>2,090</td>
<td>18,900</td>
<td>4/5</td>
</tr>
<tr>
<td>5.50</td>
<td>29.93</td>
<td>1,290</td>
<td>19,600</td>
<td>3/4</td>
</tr>
<tr>
<td>5.50</td>
<td>27.18</td>
<td>1,660</td>
<td>19,600</td>
<td>4/5</td>
</tr>
<tr>
<td>6.00</td>
<td>24.45</td>
<td>1,960</td>
<td>21,400</td>
<td>4/4</td>
</tr>
</tbody>
</table>
Fig. 11. Percent mortalities of cotton mice exposed for 10 minutes to SRM exhaust as a function of HCl concentration. Exposure to 5.24 g ($\Delta$).
Fig. 12. Percent mortalities of cotton mice exposed for 10 minutes to SRM exhaust as a function of Al₂O₃ concentration. Exposure to 5.24 g (Δ).

N = 11
LD₅₀ = 169

N = 10
LD₅₀ = 173

SRM Exhaust Concentration (mg Al₂O₃/m³/g body weight)
exposure to 5.24 g which was previously mentioned. The LD<sub>50</sub>'s for both HCl and Al<sub>2</sub>O<sub>3</sub> were determined from the regression lines (Figs. 11 and 12). The LD<sub>50</sub> for HCl was 52 ppm/g of body weight (N = 10) and 56 ppm/g of body weight (N = 11). The LD<sub>50</sub> for Al<sub>2</sub>O<sub>3</sub> was 173 mg/m<sup>3</sup>/g of body weight (N = 10) and 169 mg/m<sup>3</sup>/g of body weight (N = 11).

**External Observations of Exposed Mice**

In all instances, exposure to the SRM exhaust caused signs of respiratory distress and dyspnea in the mice. Exposed animals showed small accumulations of particulate matter on the fur and a small amount of moisture on the nose. Gum bleeding and excess amounts of saliva were observed occasionally. Gross internal observation showed the lungs of normal animals to be a pinkish color with no spots, while lungs of exposed animals were darker in color but not brown. The pulmonary blood vessels of exposed animals were quite distended and the right side of the hearts were engorged with blood.

**Histological Observations of Exposed Mice**

Microscopic observations of normal lung and tracheal tissue showed the cotton mouse to be remarkably free from the various respiratory diseases common to many mice, both wild types and lab stock. A few of the normal lungs exhibited a slight focal peribronchiolar lymphoid hyperplasia and a few showed a very slight focal hemorrhage. Mononuclear cells were present but in very insignificant numbers (report dated November 1975 from Dr. T. E. Murchinson of Dawson Research Corporation, Orlando, Florida).
Trachea and lung tissue from 10 animals which died during a 10 minute exposure to SRM exhaust were examined by Murchinson (personal communication, 1975). A summary of his study is presented in Table 8. There were no apparent changes produced in any of the tracheas. Eight of the animals had lung tissue which showed slight changes of the type associated with early inflammatory reactions. Five of the animals had pulmonary lesions which could possibly be attributed to the exposure. The lesions were of two types: margination of leukocytes and acute peribronchiolitis. Animals 789 and 848 showed very slight changes, 733 and 782 showed slight changes and 833 showed moderate changes. The five animals which showed these lesions were exposed to the highest concentrations of HCl and to some of the higher concentrations of Al$_2$O$_3$.

No signs of significant particulate matter (Al$_2$O$_3$) accumulation were observed in the tissues examined.
Table 8. The effects of various lethal concentrations of HCl and Al₂O₃ on lung tissue of the cotton mouse. All tissue was obtained from mice which died during a 10 minute exposure to SRM exhaust (Murchinson, personal communication, 1975).

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Experimental Concentration HCl (ppm)</th>
<th>Experimental Concentration Al₂O₃ (mg/m³)</th>
<th>Lung Tissue Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>825</td>
<td>1170</td>
<td>5090</td>
<td>Very slight focal hemorrhage</td>
</tr>
<tr>
<td>851</td>
<td>1170</td>
<td>5090</td>
<td>Focus of very slight subacute pneumonia* and very slight hemorrhage</td>
</tr>
<tr>
<td>778</td>
<td>1290</td>
<td>3500</td>
<td>Very slight focal hemorrhage and moderate congestion</td>
</tr>
<tr>
<td>814</td>
<td>1290</td>
<td>3500</td>
<td>None</td>
</tr>
<tr>
<td>761</td>
<td>1660</td>
<td>5620</td>
<td>None</td>
</tr>
<tr>
<td>733</td>
<td>1660</td>
<td>5620</td>
<td>Slight focal margination of leukocytes; slight focal acute peribronchiolitis</td>
</tr>
<tr>
<td>833</td>
<td>1660</td>
<td>5620</td>
<td>Marked margination of leukocytes in pulmonary vessels; slight acute peribronchiolitis</td>
</tr>
<tr>
<td>789</td>
<td>1960</td>
<td>5060</td>
<td>Focal, very slight margination of leukocytes and acute peribronchiolitis</td>
</tr>
<tr>
<td>848</td>
<td>1960</td>
<td>5060</td>
<td>Moderate congestion; very slight acute peribronchiolitis</td>
</tr>
<tr>
<td>782</td>
<td>2090</td>
<td>5910</td>
<td>Slight margination of leukocytes; very slight acute peribronchiolitis</td>
</tr>
</tbody>
</table>

*The pneumonia may have been a preexisting condition.
DISCUSSION

The Extrapulmonary Apparatus

There is one significant difference between the tracheal dimensions of the Florida mouse and the cotton mouse. The external length of the trachea of the Florida mouse appears to be longer (approximately 0.57 mm) than that of the cotton mouse (p < 0.05). All other external measurements are the same. The internal tracheal lengths, however, show no significant difference and are more reliable measurements owing to the ease of locating the cricoid cartilage on a slide section rather than on the entire preserved trachea. The coefficients of variation are relatively low for all dimensions and indicate only a small amount of variance within each species (Tables 3 and 4).

Only previous study of respiratory tree dimensions of either species studied herein was by Washington (1976). In his study of the Florida mouse Washington found the tracheal and lumen diameters to be much larger than those in this study. Washington's tracheal and lumen diameters were 37.8 and 27.8 mm, respectively. The columnar cell height determined by Washington was 5.5 μm, less than 1/2 the value in my study. The cilia height (2 μm) in the Washington study was about 1/2 that determined in my study. The lack of agreement between the two studies may have resulted from the use of different micrometer techniques.

Studies on other rodents similar in size to the Florida and
cotton mouse seem to indicate that the values determined in my study are within an acceptable range. The kangaroo rat (Dipodomys merriami) has an internal diameter of 0.9 to 1.1 μm, columnar cell height of 15 to 18 μm, and cilia height of 7.6 μm (Babero et al., 1973). The Swiss mouse (1 to 3 weeks old) has a columnar cell height of 20 to 22 μm (Hansell and Moretti, 1969).

The cellular structure of the tracheas of both species is similar to that of most vertebrates; however, a few differences were noted. Hansell and Moretti (1969) reported non-ciliated columnar cells in large numbers in Swiss mice and suggested that in the absence of goblet cells they function in mucin production. Goblet cells appeared to be absent in the Florida and cotton mouse; however, the small number of gaps between some columnar cells could be spaces where previously existing goblet cells were destroyed during slide preparation. The absence of goblet cells in the Florida mouse agrees with the findings of Washington (1976). The kangaroo rat and desert wood rat (Neotoma lepida) have no goblet cells (Babero et al., 1973), thus indicating that the lack of goblet cells is not uncommon among rodents. Hummel et al. (1966), however, reported many goblet cells in the tracheal epithelium of the lab mouse (Mus).

Riad (1960) suggested that the absence of mucus secreting structures would represent a mechanism for water economy. Glenn (1970) found that on a weight specific basis, Peromyscus consume more water than expected. Glenn showed that the non-burrowing cotton mouse becomes quite dehydrated in the field, thus the absence of
goblet cells could be an adaptation for water conservation. The Florida mouse nests in burrows and should be more hydrated due to the high humidity in the burrows. Absence of goblet cells would seem unnecessary unless the warm habitat of the Florida mouse requires water economy adaptations. Both species examined in the study exhibited numerous seromucous glands in the anterior region of the tracheas which should provide for water loss. The presence of these glands in the Florida mouse was demonstrated by Washington (1976). Roe and Walters (1965), however, stated that mice possess virtually no tracheal mucus-secreting glands and rely entirely on surface goblet cells for providing mucus.

Mononuclear cells are reported to be present in the tracheal epithelium of most mammals and the Florida and cotton mice are no exception. Washington (1976) confirmed their presence in the Florida mouse.

The Intrapulmonary Apparatus

The bronchiolar and alveolar dimensions of the Florida mouse and cotton mouse do not differ significantly. The coefficients of variance are quite low for all structures measured with the exception of the atria diameter of the Florida mouse which was measured between the inner edges of the alveoli (Tables 5 and 6).

Only two studies have been located in which bronchiolar structures were measured in rodents. In their study of desert rodents, Babero et al. (1973) found the kangaroo rat to have both proximal and distal lumen diameters much larger than either the Florida or cotton mouse. Dimensions of larger rodents such as desert
wood rat, antelope ground squirrel (*Ammospermophilus leucurus*), and laboratory white rat (*Rattus norvegicus*) were somewhat closer in value but still larger. Washington (1976) found the Florida mouse to have a smaller diameter (31 to 39 \( \mu \text{m} \)) but did not differentiate as to proximal or distal regions. The terminal columnar epithelial cells measured herein are within the range listed for the kangaroo rat, but larger than the 5 \( \mu \text{m} \) size reported by Washington (1976).

The presence of cuboidal cells in the terminal bronchiolar epithelium was not noted by Washington (1976); however, the height of 8.24 \( \mu \text{m} \) for the Florida mouse in my study is close to the columnar cell height of 5 \( \mu \text{m} \) in Washington's study. The kangaroo rat contains both types of epithelial cells (Babero et al., 1973). The small amount of cilia on the epithelial cells contradicts the findings in the Florida mouse (Washington, 1976) and several desert rodents (Babero et al., 1973). The smooth muscle layer was thicker than that of the Florida mouse in Washington's study (1976).

Goblet cells were not observed in the bronchioles of the Florida or cotton mouse, nor have they been reported in the bronchioles in any rodent studies (Washington, 1976; Babero et al., 1973; Reith and Ross, 1970; Hummel, et al., 1966). Mononuclear cells are found in most rodent bronchioles.

The parts of the alveolar apparatus are quite similar in size for the Florida and cotton mouse. Values for the Florida mouse in my study were much larger than those determined by Washington (1976); however, my values correspond well with the ranges for other rodents (Babero et al., 1973). Reith and Ross (1970) reported the smallest
alveoli (30 μm) in mammals to be found in bats and shrews; however, Washington (1976) measured the Florida mouse alveoli to be 11 to 14 μm. Of some interest are the dimensions for the atrial diameter as no distinction was made in any literature as to the standards used for measuring. The measurements could be for the lumen or for the distance between the far walls of two alveoli on opposite sides of one atria. The cell types for both species are similar to those in most rodents with the exception of the few cuboidal cells which project from the walls of some alveolar ducts and atria. The large variety of alveolar shapes is characteristic of several rodent species (Babero et al., 1973).

The anatomical differences between species may represent morphological adaptations to different environments or they could result from differing phylogenies (Babero et al., 1973). The present study indicates no significant difference between the Florida mouse or cotton mouse even though the cotton mouse occupies habitats with much colder weather during the fall and winter months. It would be interesting to examine respiratory tract dimensions of cotton mice living in the most northern parts of their range to determine if intraspecific differences exist. It is doubtful that differences exist among Florida mice owing to their limited range. Phylogenetically speaking, the similarities of the two species could be expected owing to the fact that they both shared a common ancestor - *Peromyscus ochraventer* (Hsu and Arrighi, 1968).

Exposure Statistics

The theoretical concentrations of the SRM exhaust differed
from the experimental concentrations because of the dewing of the
SRM exhaust on the chamber walls (Madsen, 1974b). Room humidity
varied from 45 to 65 percent and thus aided the absorptive process.

All data concerning correlation between percent mortality and
concentration of HCl and Al₂O₃ shows the higher degree of correlation
to exist when the questionable exposure was omitted. The regression
lines for HCl concentration (N = 10 and N = 11) appear steeper than
the regression line shown by Higgins et al. (1972) for HCl alone.
This could indicate synergistic action between HCl and particulate
Al₂O₃ in producing mortalities.

No regression lines or LD₅₀'s for Al₂O₃ were noted in the
literature reviewed.

Owing to the movement of the SRM exhaust ground cloud and
relatively low concentration of HCl and Al₂O₃ within the cloud, it
would be difficult to show a cause-effect relationship between the
SRM exhaust and mortality of cotton mice near the launch area. The
chance for exposure to a ground cloud which could cause mortality
among free ranging cotton mice appears very remote.

**External Observations of Exposed Mice**

HCl is generally regarded as a contact irritant which causes
irritation and erosion of the epithelium and mucous membrane of the
eyes and upper respiratory tract (Cicerone et al., 1973; Cicerone
et al., 1972; Rowe et al., 1971; Efimova, 1964; Machle et al., 1942;
Flury and Zernick, 1931; Haggard, 1924; NASA anon undated). No eye
damage was observed on the animals, but moisture on the noses of most
exposed animals indicated irritation of the nasal epithelium. The
occasional bleeding of gums and formation of excessive saliva also indicated irritation.

Irritation of the upper respiratory tract may stimulate several reflexes which tend to prevent the deeper penetration of the irritant. Rowe et al. (1971) and Haggard (1924) list these reflexes as coughing, constriction of the larynx, closure of the glottis, and inhibition of respiration. Owing to the inability to see through the SRM exhaust cloud, observations on the presence or absence of these reflexes was impossible.

The somewhat darker color of the exposed lungs could indicate a very slight amount of edema or hemolysis, but not enough to contribute to death (Murchinson, personal communication, 1975). The large amount of blood in pulmonary blood vessels and hearts could indicate blood stagnation with the result of body tissue hypoxia.

**Histological Observations of Exposed Mice**

The establishment of the cotton mouse as being relatively free from respiratory diseases was fortunate as many rodent species are quite susceptible to various respiratory infections. Normal histology was studied in order to determine the usefulness of the cotton mouse in this research. Murchinson (personal communication, 1975) found some of the normal lungs to have focal peribronchiolar lymphoid hyperplasia which resulted from spontaneous murine pneumonia. This inflammatory condition is said to be common in mice colonies and some researchers indicate the condition to be a normal species characteristic in rat lungs (Innes et al., 1967). The slight focal hemorrhage could have been merely a result of death.
The presence of mononuclear cells is documented in most rodent species (Innes et al., 1967; Hummel et al., 1966; Karrer, 1956).

Changes in the respiratory tree were slight, but this could be expected from such a short exposure time (10 min). Since the trachea showed no signs of tissue damage, the lack of serious damage in the lungs was expected. Machle et al. (1942) indicated that the irritation resulting from HCl inhalation can reach the alveolar epithelium, but only later during infection after upper passageways had been affected. Lower HCl concentrations (60 ppm) have been shown to cause a loss of ciliary activity in rabbit tracheas (Cralley, 1942). The inflammatory reactions in the lungs with no corresponding tracheal damage may have resulted from some synergistic reaction between HCl and Al₂O₃.

The lung changes which occurred involved alveolar epithelial damage - those of a type relating to inflammation, not death. Margination of leukocytes is one of the earliest types of inflammatory reactions exhibited by lung tissue. The margination was not diffuse enough, however, to have affected gas exchange. Acute peribronchiolitis is a second stage of inflammatory reaction in which leukocytes begin infiltrating the peribronchiolar tissue. These changes alone were not enough to have been the cause of death (Murchinson, personal communication, 1975).

Inhaled particles which reach the alveoli initiate a macrophage response and if cell death occurs, phospholipid substances are released to provoke a fibrous reaction (Spencer, 1968). The 10 min exposure period would not have allowed sufficient time for fibrosis
due to $\text{Al}_2\text{O}_3$ to occur. Also, Murchinson (personal communication, 1975) observed only a very few mononuclear cells which appears to be normal for the animals.

The histological results indicate that death did not result from single action of $\text{HCl}$ or $\text{Al}_2\text{O}_3$. However, some evidence has been demonstrated for possible synergistic action of the two substances. The most likely cause of death now appears to have been a sudden shift in blood pH. A possible mechanism for death concerning pH change is given below.

Upon inhalation, gaseous $\text{HCl}$, alone or adsorbed to particulate $\text{Al}_2\text{O}_3$, overwhelmed the blood buffer system and metabolic acidosis resulted. The $\text{HCl}$ would have been transported to the alveolar spaces more rapidly if it was adsorbed on small particulate $\text{Al}_2\text{O}_3$. The SRM exhaust caused only very slight edema in a few cases so the diffusion of gases ($\text{O}_2$, $\text{CO}_2$, and $\text{HCl}$) across the lung alveolar-capillary membrane was not inhibited. In order to compensate for the metabolic acidosis, increased ventilation occurred and resulted in a decreased partial pressure of $\text{CO}_2$ ($P_{\text{CO}_2}$) in the blood. This could have lead to cerebral ischemia which would have caused death within a few minutes. If this were not the cause of death, the mechanism would have continued with the decreased $P_{\text{CO}_2}$ and caused distention of pulmonary blood vessels. Stagnation of blood within the lungs resulted in heart blood vessels distending and becoming engorged with blood. Both lung and heart engorgement were actually observed. The accompanying decreased cardiac output could cause tissue hypoxia and the resultant death (Mickus, personal
communication).
SUMMARY

1. Microscopic examination of the tracheal dimensions of Florida and cotton mice showed no significant difference between the two species, but external examination of tracheal lengths showed the Florida mouse trachea to be longer than that of the cotton mouse.

2. Microscopic examination of the intrapulmonary apparatus of Florida and cotton mice showed no significant difference between the two species.

3. Cotton mice exposed to SRM exhaust for 10 min demonstrated an LD_{50} of 52 to 56 ppm HCl/g body weight and an LD_{50} of 169 to 173 mg Al_2O_3/m^3/g body weight.

4. LD_{50} values suggested that SRM exhaust components may have a synergistic lethal effect when compared to the effects of individual components of the exhaust.

5. Cotton mice exposed to SRM exhaust exhibited external signs of respiratory distress and dyspnea and internal signs of early inflammatory reactions, neither of which was severe enough to cause death.

6. Sudden shift of blood pH probably caused the observed deaths of cotton mice.
LITERATURE CITED


