A MORPHOLOGIC STUDY OF ACUTE TOXICOSIS IN PARAKEETS EXPOSED TO PYROLYSIS PRODUCTS OF POLYTETRAFLUOROETHYLENE-LINED COOKING PANS

By

Roger E. Wells

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ABSTRACT

A MORPHOLOGIC STUDY OF ACUTE TOXICOSIS IN PARAKEETS EXPOSED TO PYROLYSIS PRODUCTS OF POLYTETRAFLUOROETHYLENE-LINED COOKING PANS

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Thirty-two parakeets were subjected to the fumes (pyrolysis products) from heated (400 C) polytetrafluoroethylene (PTFE)-lined and plain aluminum pans for varying periods of time using a contrived exposure apparatus. Birds exposed to lethal doses of PTFE pyrolysate (9 minutes or longer) had rapid onset of clinical signs that progressed from eyelid blinking and panting to incoordination and terminal convulsions. Pathologic changes were evaluated grossly and microscopically using light, transmission and scanning electron microscopy. Significant lesions were limited to the lower respiratory system (excluding air sacs) and consisted of pulmonary congestion, hemorrhage, and respiratory epithelial necrosis. Clinical signs and death were attributed to anoxia from the loss of gas exchange surfaces and obstructed air circulation. Sublethal doses produced less severe effects. Fumes from plain aluminum pans had no adverse effects on birds.

DEDICATION

THIS WORK IS DEDICATED TO MY UNSELFISH AND LOVING WIFE,

EARLDEAN

ACKNOWLEDGEMENTS

I wish to express my appreciation for the help and guidance of my graduate committee members, Drs. Stuart Levin, Allan Trapp and Patricia O'Handley. Dr. Levin, my major professor and residency advisor, has been my good friend and teacher for the past three years. Dr. Trapp, a good teacher and necropsy floor detective, suggested that I do this interesting project.

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I am very grateful for the time and efforts of Dr. Ron Slocombe in performing the scanning electron microscopy.

Last but not least, I would like to thank the Melopsittacus family for their help.

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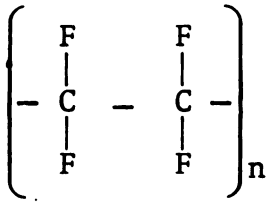
INTRODUCTION AND LITERATURE REVIEW

The plastic polytetrafluoroethylene (PTFE) is a synthetic linear fluorocarbon polymer with high molecular weight and many useful qualities. The basic building block is the gaseous monomer tetrafluoroethylene, which is represented in the polymer by the simple structural formula:



The polymer resin is formed under heat and pressure and has 10,000 to 100,000 units per molecule. Because of the extremely strong binding forces between carbon and fluorine, PTFE has relatively high thermal stability and strength, chemical and biological inertness, electrical insulating qualities, and low friction coefficients and antistick (lubricant) properties.[1,2]

Many popular uses for PTFE have evolved because of these particular traits. In some manufacturing processes, mold release sprays and parting compounds utilize the heat resistant and nonstick properties of PTFE. The modern American consumer is most familiar with the famous nonstick

surfaces of PTFE-lined cookware sold under the tradenames "Teflon" and "Silverstone".[a] Following the discovery of PTFE in 1941, over a hundred million pounds of PTFE had been produced and used by 1970.[2]

Thermal stability has permitted the use of PTFE at elevated temperatures. Few plastics can withstand temperatures above 100 C, but PTFE is rated by the manufacturer for continuous use at 260 C. Most plastics are highly flammable, but PTFE is not flammable.[2,3] The resulting high temperature use of PTFE has been a cause for concern because there is risk of high temperature abuse which results in the degradation or pyrolysis of PTFE.[2]

Intact PTFE is nontoxic and biologically inert. It has been used in human and animal tissue implants such as blood vessel and heart valve prostheses without harmful effects.[3,4] Chronic animal feeding studies have proven PTFE to be nontoxic by oral route.[2] Respiratory physiologists use aerosolized PTFE particles in human volunteers to study lung clearance.[4] While intact PTFE is nontoxic, the degradation products of PTFE pyrolysis can cause toxicosis in human beings and animals.

Most inorganic materials, like metals and ceramics, soften and lose their strength when overheated. Organic polymers, including PTFE and other synthetics, as well as natural polymers like wood, silk, cotton, and vegetable oil, undergo decomposition or breakdown of their chemical structures

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[a]E. I. duPont de Nemours & Co. (Inc.), Wilmington, DE.

when overheated.[2,5,6] These by-products of degradation, whether it be pyrolysis or combustion, are given off as fumes or gases. High temperature abuse (>260 C) of PTFE results in degradative pyrolysis rather than oxidative combustion because of the strong covalent bonds. This destruction or loss varies from about 1 x 10^{-3}%/hr at 260 C to about 4%/hr at 450 C where pyrolysis is measured as weight loss from the original sample.[2,5]

Polymer pyrolysis or combustion products pose serious public health considerations, whether the polymer is synthetic or natural.[2,5,7] One of the first indications of hazard from the decomposition of synthetic polymers was the Cleveland Clinic disaster of 1929. A fire in the nitrocellulose X-ray film storage area in the hospital produced large amounts of nitrogen oxides and carbon monoxide. The fumes spread throughout the building and killed 125 persons.[3,8] Nitrocellulose is very combustible and is never intentionally used at elevated temperature, but PTFE is noncombustible and is purposely used at high temperatures.

In 1951, 10 years after the advent of PTFE production, Harris described the first reported human cases of toxicosis due to the inhalation of PTFE pyrolysis products.[9] These first cases (2 British, 2 American) were associated with the industrial fabrication of PTFE products at temperatures of 350 to 450 C. Harris compared the transient symptoms of chest tightness and pain, cough, sore throat, and pyrexia to those of metal-fume fever and brass-founder's ague, which were 2 well-recognized occupational illnesses.[10] At the time

of this report, very little analytical work had been done, but it appeared that the toxic principle was "an extremely fine sublimate liberated from the polymer and carrying absorbed hydrofluoric acid." The new syndrome was given the name polymer-fume fever.

Since Harris' first report, a number of papers on polymer-fume fever have been published.[3,11-18] Most polymer-fume fever cases are related to occupational exposure. Smoking of tobacco contaminated with PTFE has been the most common source of PTFE pyrolysis products. As little as 0.4 mg of PTFE on a cigarette can cause illness. The spectrum of signs and symptoms is essentially the same as described by Harris, but dizziness, nausea, and radiographic evidence of pulmonary edema are often included.[14,17] Few victims have all of the signs. The clinical signs are usually delayed for at least 1 hour after exposure and persist for 24 to 48 hours. No fatalities have been reported and the syndrome is considered to be benign.[14] One victim, with a previous 9-month history of more than 40 separate episodes of polymer-fume fever, had diffuse pulmonary fibrosis diagnosed at autopsy. Her death was attributed to the rupture of a cerebral aneurysm considered unrelated to polymer-fume fever.[11,18]

Mass spectrometry, infrared absorption spectrometry, gas chromatography, and wet chemistry analysis have been used to identify the various compounds in the pyrolysis products.[1-3,5,19-22] Pyrolysis of PTFE in room air can produce at least 9 different fluorinated compounds. These

are: monomeric tetrafluoroethylene, hexafluoroethane, hexafluoropropylene, octafluorocyclobutane, perfluoroisobutylene, carbon tetrafluoride, oxygen difluoride, carbonyl fluoride, and hydrogen fluoride. Free fluorine has never been found. The composition and concentrations of compounds in PTFE pyrolysis products vary with the pyrolysis temperature, but time, humidity, atmospheric oxygen tension, and even the size and shape of the original plastic are also determining factors. Only 3 of the 9 compounds that have been identified and quantitated are considered to be the principal intoxicants.[1-3,5,19-23] Studies using isolated compounds have shown that carbonyl fluoride, hydrogen fluoride, and perfluoroisobutylene are the major toxic products. These compounds are usually present in significant quantity and have individual toxicities that merit this distinction.

Perfluoroisobutylene is found in low concentrations compared to carbonyl fluoride and hydrogen fluoride and is 10 times as toxic to rats as deadly phosgene gas.[20] Hydrogen fluoride is a strong acid. Carbonyl fluoride reacts with water on moist surfaces (e.g., lung tissue) to form hydrogen fluoride and carbon dioxide.[22] The concentration of carbonyl fluoride, when measured indirectly as the total hydrolyzable fluoride, appears to correlate well with the degree of toxicity of a pyrolysate.[1,19,22]

Another important characteristic of PTFE pyrolysis products is that they are made up of both gaseous and particulate materials. This was suggested in the very first publication on polymer-fume fever (Harris).[9] Particulate

fumes are found whenever PTFE is heated to a temperature at which weight loss occurs.[5] Transmission electron microscopy of collected particles has shown that most (96%) of the particles are less than 1 μm in diameter, with none larger than 3 μm in diameter.[5,6,19] The size is well within the respirable range.[4] The exact composition of the particles is unknown, but they are made up of fluorinated compounds. Pyrolysis products can be effectively filtered with membrane filters having 0.20 to 0.45 μm pore diameters. After filtration the pyrolysate is nontoxic to laboratory animals.[5,19,20] This suggests that the particles are toxic themselves or they carry absorbed toxic compounds such as carbonyl fluoride, hydrogen fluoride, or perfluoroisobutylene. This gaseous and particulate vapor is not evolved as a visible cloud or smoke.[24]

The toxicology of PTFE pyrolysis is a confusing arena. Problems arise because of the inherent complexity of inhalation exposure toxicology and the variability in composition of pyrolysis products. In 1968, MacFarland published a discussion of the problems incurred in defining the toxicity of pyrolysis products of plastics.[22] The major difficulty lies in establishing a quantitative dose-response relationship in the test system. In the case of PTFE pyrolysis, a number of questions must be answered. Some of these are listed:

1. What is the form, shape, and size of the sample that is to be pyrolyzed?

2. What is the pyrolysis temperature?

3. What is the composition of the gas atmosphere in which the pyrolysis is done?

4. How will the amount of pyrolysis products be measured?

5. How will the pyrolysis products be delivered to the subjects of the exposure?

6. What are the temperature and humidity of the exposure chamber?

7. How much of the delivered products is inhaled and retained by the subjects?

8. What is the composition of the delivered pyrolysis products?

9. Which of the pyrolysis products are toxic?

10. What will be the duration of exposure and observation periods?

Because of the obvious technical problems confronted in trying to answer some of these questions, the published animal toxicology studies have varied in methods and results. But there is complete agreement that PTFE pyrolysis products are toxic to all animals studied. The following species have been used in experimental inhalation exposure to PTFE pyrolysis products: squirrel monkeys, dogs, cats, rabbits, guinea pigs, rats, mice, quail, chickens, parrots, and parakeets.[2,5,9,20,24-27] Toxicosis and death are readily produced but no animal has been found to respond in exactly the same manner as human beings with polymer-fume fever. Thus, no animal model exists for the human illness.

In animal studies, the most common approach has been to find the approximate lethal temperature (ALT). This is the temperature at which there is 100% lethality in a group

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exposed to the pyrolysis products at a constant exposure (flow) rate for 2 or 4 hours.[1,2,20,23,26] The ALT's for any number of species, age groups, breeds, or sexes can be compared if all factors except the subject are constant. The ALT does not establish a lethal dose in terms of quantity of pyrolysis products, but it does provide a means to repeat or compare experiments. The ALT has proved to be a useful measurement.

Another method of determining an approximation of exposure dose (lethal or sublethal) has been to measure the loss in weight of the original sample that is pyrolyzed.[20,23] The dose is reported in weight but this assumes that all of the pyrolysis products are delivered to the subject of the exposure. Significant condensation of the pyrolysate in the delivery system and other losses have been reported.[6,19,24] Lethal concentrations (LC_{50}), approximate lethal concentrations (ALC), and lethal doses (LD_{50}) with 30-minute and 1-hour exposures have been calculated.[20,23,24]

Most animal studies have reported on the acute ($\leq$24 hours) and delayed (24 to 48 hours) lethality of PTFE pyrolysis products in rats.[1,9,20,23,24] The toxic effects in survivors of exposure have been followed for as long as 3 weeks postexposure.[20,23] Only 2 reports have mentioned clinical signs observed in toxic exposures.[9,25] One of these studies involved the exposure of birds, guinea pigs, and mice in field simulations with heated (180 to 500 C) PTFE-lined cooking pans in a small room (20 m^3) containing the animals. The clinical signs in the parrots, chickens, and parakeets

were described as initial excitement and unrest that progressed to apathy, incoordination, and death. The guinea pigs and mice were unaffected. The other study reported respiratory "irritation", dyspnea, sluggishness, and "illness" in exposed rats. All other reports referred to the animals as either dead or alive and made no comments on clinical behavior or signs of illness.

The most consistent acute gross lesions in all animal species studied have been severe pulmonary congestion, edema, and hemorrhage.[1,9,20,23-25] Ersham reported lung edema and congestion, visceral congestion, and myocardial degeneration as the significant macroscopic changes in birds.[25] The gross lesions are nonspecific but are so consistent that the lung appears to be the primary target organ on macroscopic examination of animals killed in experimental exposures.

Histologic examination of lung tissues from mammals exposed to lethal doses of pyrolysate has shown acute lesions typical of pulmonary irritants.[24] The tissue response has been characterized by alveolar cell swelling and desquamation, blood capillary damage, and edema and hemorrhage into the airways and interstitium. Avian species are similarly affected. Mild degenration of liver, heart, and kidney tissue is mentioned in several reports but is an inconsistent finding.[5,23] Cellular inflammatory infiltrates are not seen in most acute studies. Neutrophils, lymphocytes, and macrophages, along with alveolar epithelial cell hyperplasia and fibroplasia have been found in chronic survivors of sublethal doses of pyrolysate in rats.[23] Several papers have reported

the finding of crystalline material in the lung parenchyma and have suggested that this may represent the toxic particulates.[1,23]

Parakeets and quail have been reported to be the most susceptible of all species studied in experimental PTFE pyrolysis toxicosis.[5,26] This sensitivity of small birds to inhaled materials was not surprising. For ages, caged canaries have been carried into mines as sentinels to low oxygen or toxic gases. When the canaries died the miners wasted no time in leaving the mineshaft! There are 2 papers that report the increased susceptibility of parakeets (and quail) in comparison to mammals, including human beings. Ersham showed that parakeets succumbed to the fumes from a heated (180 to 500 C) PTFE-lined pan while guinea pigs and mice in the same room did not have clinical signs of toxicosis.[25] The other report compared the 4-hour ALT's of parakeets and quail, 260 C and 280 C, respectively, with the 450 C ALT of rats.[26] The birds were killed at a much lower pyrolysis temperature.

Only 2 reports of natural incidents of animal toxicosis due to PTFE pyrolysis were found in the literature. In Switzerland, Ersham reported 2 cases involving the loss of pet caged birds shortly after empty PTFE-lined pans were overheated.[25] In the first case, 2 parakeets and 1 finch died. In the second case, 45 out of 65 tropical birds died. The lesions of pulmonary congestion and edema, brain hemorrhage, and hepatic and myocardial degeneration and necrosis were found. The owner with the greater loss of birds observed

terminal convulsions in some of the birds. In England, Blandford published a case report on the death of 5 cockatiels and polymer-fume fever in the owner of the birds.[16] A PTFE-lined water pan had boiled dry while unattended for 15 minutes. Five birds, in an adjoining room, died within 20 to 30 minutes after the owner discovered the overheated pan. The birds had dyspnea and vomited before death. After another 30 minutes, the owner became ill with shortness of breath, paroxysmal coughing, tightness of the chest, chills, and headache. These symptoms of polymer-fume fever lasted for about 24 hours. Another person who was in the same room as the birds, but had not gone near the overheated pan, had no complaints of illness. Blandford reported the gross lesions of pulmonary congestion and edema, visceral congestion, and darkened venous blood. Microscopically, the lungs were congested and hemorrhagic. The epithelium lining the atria (sacculations of the secondary bronchi) was disrupted. Crystalline (carbonaceous) deposits were seen in the lung parenchyma. The livers were congested but did not have the degeneration or necrosis as reported by Ersham.

After PTFE-lined pans had been on the market for several years, the manufacturer's toxicology and safety laboratory[b] began to get sporadic reports of death in pet birds following the thermal abuse of the pans. This prompted the parakeet

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[b]Haskell Laboratory for Toxicology and Industrial Medicine, E. I. duPont de Nemours & Co. (Inc.), Wilmington, DE.

and quail study using several types of pans, cooking temperatures, and cooking media (oils).[26] Normal open pan cooking (frying) temperatures range from 130 to 280 C. Some specific temperatures at which various foods are cooked are listed:[5,26]

		° C
1.	Fish fillet	130
2.	Fried noodles with fat	180-190
3.	Fried doughnuts	165-170
4.	Fried veal cutlets	150-195
5.	French fries	196
6.	Fried eggs	<190
7.	Fried meat	≤280

Butter and corn oil will flash or burn at about 280 C. This flash point and the normal cooking temperature range suggested that the high temperatures necessary for toxic PTFE pyrolysis are not normally attainable in cooking with PTFE-lined pans. In fact, the burning foods and melting plastic handles were lethal at temperatures less than 260 C.[26] Empty PTFE-lined pans were toxic and lethal at temperatures greater than 260 C. A neglected empty PTFE-lined pan can reach a temperature of 400 C in less than 8 minutes on a conventional electric stove.[c] Human exposure trials with overheated pans in use conditions did not produce polymer-fume fever.[5] Birds, especially parakeets, are much more sensitive to the thermal degradation products than human beings or rats.

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[c]Personal observation.

The manufacturer and the Food and Drug Administration have concluded that PTFE-lined cookware is safe for conventional kitchen use.[5,26,28] The products bear no labels suggesting hazards to consumer and pet animal health.

The Animal Health Diagnostic Laboratory at Michigan State University receives numerous pet caged birds for postmortem examination. Many times there is no useful history submitted with the carcass--the bird is just found dead. Sometimes a more complete history is available and includes the fact that a PTFE-lined pan has been accidentally overheated shortly before the bird died. The pan has been either boiled dry of water or the burner under an empty pan has been accidentally turned on. My own electric stove can heat an empty PTFE-lined pan to 400 C in less than 8 minutes from the time the burner is turned on. Based on published accounts of the toxicity of PTFE pyrolysis products in experimental, occupational, and household situations, it is probable that toxic amounts of PTFE pyrolysis products could be generated from neglected heated empty pans. I could find no information on the susceptibility of pet caged birds in American veterinary literature and only 2 case reports were found in European veterinary literature. The very interesting and incriminating studies on parakeet and quail sensitivity to these products were published in an industrial hygiene journal.[26] I doubt that many veterinary practitioners and diagnosticians, or bird owners, were made aware of this publication and the hazards of PTFE pyrolysis.

The purposes of this study were to produce acute PTFE pyrolysis products toxicosis in budgerigars (*Melopsittacus undulatus*), better known as parakeets; to characterize and interpret the clinical signs and the gross and microscopic lesions; and to report these findings in American veterinary literature. Although these experiments would not recreate the kitchen conditions of natural exposures, the findings should be helpful to veterinarians and pet bird owners.

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ARTICLE 1

ACUTE TOXICOSIS IN PARAKEETS EXPOSED TO PYROLYSIS PRODUCTS OF POLYTETRAFLUOROETHYLENE-LINED COOKING PANS

INTRODUCTION

Polytetrafluoroethylene (PTFE) is a synthetic polymer with the empirical formula $(CF_2CF_2)_n$. Because of its plastic nature, lubricant properties, and relative heat stability, it has many popular home and industrial uses.[1] It is the polymer resin used in the plastic lining of kitchen cookware sold under the tradenames "Teflon" and "Silverstone".[a] This coated cookware is considered to be safe at normal cooking temperatures, but may be a health hazard at higher temperatures.[1-3]

At temperatures greater than 280 C, PTFE and PTFE-coated surfaces emit degradation or pyrolysis products that are known to be toxic to human beings and animals.[1,3-8] These fumes cause a transient febrile influenza-like syndrome in human beings that is called polymer-fume fever. Accounts of the syndrome are published and have associated the illness with either exposure to high temperature PTFE fabrication or PTFE-contaminated smoking tobacco.[4,7,8,9] No animal model of the human syndrome has been established, but toxicology studies using rats, mice, guinea pigs, rabbits, dogs, chickens, quail, parrots, and parakeets have shown that these animals are susceptible to toxic PTFE pyrolysis products.[3,5,6,10] The lung is the primary target organ, with congestion, edema, and hemorrhage the only consistent pathologic changes.

The PTFE pyrolysis products have been physically and chemically characterized.[1,6,11,12] The quantity and constitution of PTFE pyrolysis products vary with the time and

temperature of pyrolysis. The principal toxic factors are particulate and have been effectively filtered with membranes of 0.2 to 0.4 mμ pore size.[5,12]

A number of fluorinated compounds have been identified in the pyrolysates, including: carbonyl fluoride, hydrogen fluoride, monomeric tetrafluoroethylene, carbon tetrafluoride, perfluoroisobutylene, and hexafluorocyclobutylene.[5,6,11] Carbonyl fluoride, perfluoroisobutylene, and hydrogen fluoride are considered to be the most toxic fractions.[5,6,11-13]

Over the years, our laboratory has received a number of pet caged birds for postmortem examination that have died within an hour of the use or abuse of PTFE-lined cookware. The famous nonstick pans can be found in almost every household in the United States, and it is likely that pet caged birds are often exposed to the toxic fumes from a neglected pan. Small birds are known to be particularly sensitive to noxious fumes.[14] For ages, canaries have been carried into mines as sentinels to toxic gases.

No reports, natural or experimental, of animal toxicosis due to these pyrolysis products could be found in American veterinary literature, but 2 clinical case reports were found in European veterinary literature.[10,15] These reports described the deaths of pet caged birds (cockatiels, parrots, finches, and parakeets) associated with the accidental overheating of empty PTFE-lined pans. One report included the findings of experimental simulations of field cases.[10] Parrots, chickens, parakeets, mice, and guinea pigs were exposed to the fumes of overheated PTFE-lined pans in a

small room. The fumes were toxic and lethal to the birds, and the significant lesions were congestion and hemorrhage of pulmonary tissue. The mice and guinea pigs had no signs of toxicosis.

The toxicology testing laboratory for the leading manufacturer of PTFE resin[b] has published an experimental study on the susceptibility of parakeets and quail to the pyrolysis products of overheated PTFE-lined pans.[3] The study was done because of reports that pet birds died following high temperature abuse of PTFE-lined pans. It is very doubtful that veterinarians and pet owners were made aware of this article in an industrial safety journal. Parakeets were found to be the most sensitive of all the species studied.

The purpose of this investigation was to experimentally produce acute PTFE pyrolysis products toxicosis in budgerigars (*Melopsittacus undulatus*), commonly called parakeets; to study and interpret the clinical signs and the gross and microscopic lesions; and to report these findings in American veterinary literature.

MATERIALS AND METHODS

Animals

Thirty-two healthy parakeets (5 to 7 months old) were purchased in one shipment from a single commercial source. The birds were of mixed color variety with 17 females and 15 males. After a 2-week period of acclimation with water, grit, and commercial parakeet seed *ad libitum*, the birds were used in a number of experimental procedures. An attempt was made

to use equal numbers of males and females in the experiments by sexing the birds according to the pigmentation of the ceres. Males usually had a light blue coloration of the ceres and females had brown ceres. At necropsy the gonadal sex of the birds was determined.

Pyrolysis Products

Polytetrafluoroethylene pyrolysis products were generated by heating PTFE-lined aluminum saucepans to 400 C on a variable temperature electric hot plate.[d] The pan dimensions were 18 cm rim diameter and 9 cm height and about 2 liter volume. The plastic handles were removed to eliminate extraneous pyrolysis products. Pan temperatures were monitored with a thermocouple indicating pyrometer[e] in contact with the center of the pan. A new pan was used for each exposure study.

Plain aluminum pans[f] of approximately the same size as the PTFE-lined pans were used for control exposures. New pans were used in the control experiments. The pans were heated to 400 C and monitored as with the PTFE-lined pans. All pans were purchased off the shelf from a local retail store and washed with soap and water before use.

Exposure Apparatus

The exposure tank (Figure 1) was a 67 liter, 77 x 32 x 32 cm, glass aquarium with a slate bottom. A partial baffle made of cardboard was taped to the inside of the tank as a means of directing the flow of gases through a stainless steel wire exposure cage (16 x 16 x 16 cm) placed in the center of

the tank. The tank was fitted with a tightly sealed but easily removed plywood lid. The lid had 5 ports. A small squirrel cage exhaust fan[g] was fastened over a 10 cm port at one end of the lid. The fan exhausted into a flexible vacuum cleaner hose (3.5 cm I.D. and 1.75 m length) and was regulated by rheostat for flow rates from 6.5 l/min to 55.4 l/min. The fan was calibrated for flow rate using a pneumotachograph,[h] transducer[i] and recorder.[j] A gas delivery tube was welded from 2.5 cm I.D. clean stainless steel tubing to form an inverted U-shape with 90° elbows joining the 27 cm, 57 cm, and 10 cm straight sections. The 27 cm arm was fitted into a port in the tank lid (at the end opposite to the exhaust fan) and extended into the tank 12 cm. The 10 cm arm was hammered onto the tapered spout of a 21 cm diameter stainless steel funnel. The delivery system was supported by a ring stand and clamps. The midpiece and the shorter arm of the delivery tube were wrapped with 48 coils of 5 mm I.D. copper tubing that was connected by rubber tubing to a cold tap water supply (10 C). This copper coil served as a heat exchanger to cool the delivery system. Three glass mercury thermometers were fitted into small ports located next to the delivery tube inlet and the exhaust port and at the center of the tank.

Birds to be exposed were put into the wire cage at the center of the tank. The tank lid was positioned on a clay-like gasket material[k] and held tightly closed with clamps. All lid ports and fittings were sealed with the same gasket material. The thermometer positions were adjusted to measure

temperatures at the delivery tube inlet, wire exposure cage, and exhaust port. During the experiments, tank temperatures at all 3 positions ranged from the ambient room temperature of 22 C to a maximum of 29 C after 65 minutes of 6.5 l/min flow from a pan heated to 400 C.

The PTFE-lined and plain aluminum pans were preheated on the electric hot plate to 400 C before exposure was started. Center-of-pan temperature was monitored with the thermocouple pyrometer. When the pan center reached 400 C, the pan and hot plate were moved to an adjustable height platform next to the exposure tank and under the collecting funnel. The platform was adjusted so that the rim of the pan was 2.5 cm from the rim of the funnel and directly beneath it. Exposure time was measured starting when the pan was correctly positioned and the exhaust fan was in operation at the proper flow rate. Room air was drawn over the heated pan, mixed with the pan fumes, and carried into the delivery system and exposure tank by the evacuating (negative) pressure of the exhaust fan (Figure 2). Exposure time was stopped as the lid was quickly opened and the small exposure cage was removed and taken to another room or when all the birds in the group were dead. Death was assumed when all body motions ceased.

Necropsy Procedure

Birds surviving exposure were humanely killed by cervical vertebral dislocation and necropsy immediately performed. Those killed by exposure had necropsies performed at the end of exposure for that particular group of birds. The carcasses

were weighed and complete gross postmortem examinations were done. Eyes, brain, heart, skeletal muscle, abdominal air sacs, proventriculus, gizzard, gonads, spleen, small intestine, liver, and kidney were collected for histologic examination.

Light Microscopy

Tissues for light microscopy were fixed in either 10% buffered formalin or 1:4.5 buffered Karnovsky's solution.[16] The fixed tissues were dehydrated, paraffin-embedded, sectioned at 6 μm thickness, and stained with hematoxylin and eosin according to a well recognized method.[17] Part of the lung tissue fixed in Karnovsky's solution was postfixed overnight in a cacodylate buffer and 2% osmium tetroxide (1:1), Epon-embedded, sectioned with glass knives at 1 μm thickness, and stained with toluidine blue.

Transmission Electron Microscopy

The Karnovsky's-fixed, Epon-embedded lung tissues were sectioned with glass knives at 900 nm thickness, stained with uranyl acetate and lead citrate, and examined using a transmission electron microscope.[1]

Exposure Procedure I

A total of 10 birds were exposed to the pyrolysis products from a heated (400 C) PTFE-lined pan until death occurred. One bird was exposed to a flow rate of 55.4 l/min. Three groups consisting of 1 bird, 6 birds, and 2 birds were exposed to a flow rate of 6.5 l/min. (At 6.5 l/min the exhaust air flow was barely perceptible to moist skin and

caused only a gentle flutter of tissue paper held against the hose.)

Exposure Procedure II

Six birds were exposed to the pyrolysis products from a heated (400 C) PTFE-lined pan for a period of 9 minutes at a flow rate of 6.5 1/min.

Exposure Procedure III

Four birds were exposed to the pyrolysis products from a heated (400 C) PTFE-lined pan for a period of 5 minutes at a flow rate of 6.5 1/min.

Exposure Procedure IV

Six birds were exposed to the pyrolysis products collected from a heated (400 C) plain aluminum pan and delivered at a flow rate of 6.5 1/min through the stainless steel tubing and funnel used in the previous 3 experiments. (The tubing and funnel were rinsed with running water between experiments.) The exposure lasted 43 minutes (or until all birds were dead).

Exposure Procedure V

Six birds were exposed to the pyrolysis products collected from a heated (400 C) plain aluminum pan and delivered through the cleaned stainless steel delivery system. To clear the previously accumulated PTFE pyrolysis products from the delivery system, the tubing and funnel were heated to bright cherry red color (about 1100 C) with propane torches for 1 hour. The tubing was connected to a

strong vacuum to remove any pyrolysis products generated during the heating process. The tubing and funnel were then scrubbed with soap and abrasive cleaning powder before a final flush with hot water. This exposure with the clean stainless steel delivery system lasted 65 minutes.

RESULTS

The experimental results are summarized in Table 1.

Clinical Signs

Exposure Procedure I

The 1 bird (Bird W) exposed to PTFE pyrolysis products at a flow rate of 55.4 1/min exhibited signs of toxicosis after 30 seconds of exposure. The bird was incoordinated, with rocking and bobbing motions. After 1 minute, the bird was down on its side and had rapid and increased respirations (panting). A terminal struggle or convulsion followed quickly and continued until the bird was dead after 3 minutes of exposure. Because of the rapid onset of clinical signs and death at the 55.4 1/min flow rate, the rest of the exposures were at 6.5 1/min, which is about 1 order of magnitude less flow. The other 9 birds in Exposure Procedure I (Birds G, 1-6,13,14) exposed to the 6.5 1/min flow rate died at 17 to 27 minutes after exposure to PTFE pyrolysis products. Clinical signs of toxicosis were varied and began with eyelid blinking at 8 to 10 minutes of exposure and progressed to open-beak panting, cage wire biting, incoordination, wing stretching and flapping, and chirping and usually

ended in a terminal struggle or convulsion, with the birds down on their sides or backs. Only 1 bird (Bird 5) died without a terminal struggle.

Exposure Procedure II

The 6 birds (Birds 7-12) exposed to PTFE pyrolysis products for a period of 9 minutes exhibited eyelid blinking and panting within 6 to 7 minutes after exposure, but no other clinical signs were observed during the exposure. Five of the birds died within 11 hours postexposure. Three birds (Birds 7, 8 and 9) died at 10, 40 and 60 minutes and 2 died unattended (Birds 10 and 12) between 2 and 11 hours after exposure. One bird (Bird 11) survived 24 hours postexposure. The first death (Bird 7) was not preceded by severe clinical signs. The other birds (Birds 8-12) were panting, chirping, and moving about the cage in an uneasy or anxious manner. Within 15 minutes of exposure, 2 birds (Birds 8 and 9) had severe signs of incoordination, lateral recumbency, and rapid noisy (click) breathing that immediately preceded death. At 2 hours postexposure, 1 bird (Bird 10) of the 3 remaining live birds (Birds 10, 11 and 12) had mildly labored breathing, but the other 2 birds were normal. The latter 2 birds (Birds 10 and 12) died during the night. At 11 hours postexposure, the 1 surviving bird (Bird 11) had mucoid feces pasted on its vent and mildly labored breathing. This bird was killed at 24 hours postexposure.

Exposure Procedure III

All 4 birds (Birds 15-18) exposed to PTFE pyrolysis products for a period of 5 minutes survived 24 hours post-exposure without exhibiting clinical signs of toxicosis during or after the exposure.

Exposure Procedure IV

All 6 birds (Birds 19-24) exposed to the pyrolysis products collected from the heated plain aluminum pan and delivered through the "dirty" stainless steel tubing and funnel used in the previous 3 procedures died at 26 to 43 minutes of exposure. The clinical signs exhibited by these birds ere the same as in Exposure Procedure I but were delayed and the terminal struggle was less severe. Eyelid blinking began at about 15 minutes and incoordination with rocking and bobbing motions began at about 25 minutes. The suspect tubing and funnel had a brownish-gray light powdery coating on the interior before being cleaned with propane torches and scrubbing.

Exposure Procedure V

All 6 birds (Birds 25-30) exposed to the pyrolysis products from the heated plain aluminum pan and delivered by the "clean" stainless steel tubing and funnel survived the 65-minute exposure period without exhibiting clinical signs of toxicosis. Three of the birds (Birds 25-27) were killed immediately after the exposure. The other 3 (Birds 28-30) were allowed to survive for 24 hours postexposure before being killed.

Gross Lesions

Mild incidental lesions were found in the liver, spleen and kidney tissues of some birds in all groups (I-V).

Exposure Procedure V

There were no gross lesions in the birds from this exposure. The lungs were pink and light in consistency. The prominent superficial tertiary (3°) bronchi (parabronchi) could be seen as linear patterns radiating from the medial aspect of the lungs (Figure 3). Blood from these birds was bright red, as if well-oxygenated. The lungs floated in the fixative solutions.

Exposure Procedures I, II and IV

These birds all had lung lesions and most had very dark discoloration of the blood (poorly oxygenated). The lungs were dark red, congested, and hemorrhagic. No internal structures such as 3° bronchi could be seen (Figure 4). These lungs sank in the fixative solutions. The most severe lesions were in birds that died during or shortly after exposure (Birds 1-10, 12-14, 19-24).

Exposure Procedure III

These birds survived 5-minute exposure to PTFE products and had similar but milder lesions than the birds of Exposure Procedures I, II and IV. Pulmonary congestion and hemorrhage ranged from focally severe to mild or moderate and diffuse (Figure 5). These lungs floated in the fixative solutions.

Light Microscopy

Significant and consistent histologic changes were restricted to the lung tissues of birds exposed to PTFE pyrolysis products.

Exposure Procedure V

The lung tissues of the 6 birds (Birds 25-30) exposed to the aluminum pan pyrolysis products were histologically normal. Figures 6 and 7 illustrate the open or lace-like appearance of normal functional lung. Figure 6 has prominent longitudinal sections of 3° bronchi. The cross section of a 3° bronchus and the radiating air capillary networks that surround it are featured in Figure 7. The walls of 3° bronchi were relatively thin with a featureless flattened epithelium and narrow bands of smooth muscle. The atria (infundibula) were seen as small sacculations in the bronchial walls (Figures 8 and 9). Air capillaries were thin walled and surrounded by blood capillaries (Figure 9). These tissues served as controls.

Exposure Procedures I, II and IV

All 22 birds in these PTFE pyrolysis exposures had severe and widespread congestion and hemorrhage of the lung tissues. Secondary (2°) and 3° bronchi were filled with blood and the lung parenchyma was dense with congested blood vessels obliterating the air capillaries (Figures 10 and 11). In longitudinal and cross-sectional views, most 3° bronchi had reduced lumen diameters and the smooth muscle bands were thickened, as if in a contracted state (Figures 10, 11 and 12).

The epithelium of the 3° bronchi was swollen, vacuolated, and disrupted (Figures 12, 13, and 14). Hemorrhagic exudate filled the bronchial lumens and congestion and hemorrhage obscured the air capillaries (Figures 13 and 14). Edema was limited to subepithelial areas of damaged bronchial walls (Figure 14). Significant mucus accumulation was present on the ciliated epithelium in 2° bronchi of 1 bird (Bird 11) that survived 24 hours postexposure in Exposure Procedure II (Figure 15). Lungs of most of the birds had small deposits of yellow-brown round to elongate particulate foreign matter within and without engulfing macrophages (not illustrated). The particles were found deep in the longitudinal folds of 2° bronchial mucosa or immediately subjacent to the flattened 3° bronchial epithelium. Similar particles were found in several of the control birds but were much fewer in number. The particles were approximately 0.5 to 1.0 x 2.0 to 3.0 μm in size.

Exposure Procedure III

These birds exposed to a sublethal dose of PTFE pyrolysis products followed by 24-hour survival had obvious lung lesions similar to but not as severe as the birds in the lethal exposures. Focal and sometimes locally extensive areas of the lungs were severely hemorrhagic and congested, but other areas were only mildly affected or normal (Figure 16). Most of the lung was open and had functional appearance.

Transmission Electron Microscopy

The control birds in Exposure Procedure V had normal appearing lung tissues at the magnifications provided by transmission electron microscopy (Figures 17, 18 and 19). The 3° bronchial epithelium was flat and featureless on the surface and had elongated dense nuclei (Figure 17). Only membranous or type I pneumocytes were seen on the surface of 3° bronchi in these particular thin sections. Type II pneumocytes with microvilli and osmiophilic lamellar inclusions were not present. This was thought to be due to chance, since birds normally do have type II pneumocytes in 3° bronchi. The air capillaries consisted of numerous round or elongated open spaces lined by intact electron-dense single-layered membranes. The epithelial cell bodies of air capillaries (type I pneumocytes) were difficult to locate and identify but, when found, were usually "corner" cells with plump triangular nuclei (Figure 18). The blood capillary networks surrounded air capillaries and the endothelium of the blood capillary was adherent to or seen as part of the air capillary wall. The endothelial membrane was less dense but thicker than the air capillary epithelial membrane (Figures 18 and 19). Erythrocytes were found in the blood capillaries but were not packed into them (not congested). Plasma proteins were seen as a finely stippled density in the blood capillary lumens. Air capillary lumens were completely empty or lucent.

In sharp contrast with the control lungs, the tissue from lethally exposed birds was abnormal, with necrosis,

hemorrhage, and disruption of architecture (Figures 20 through 23). The 3° bronchi had hemorrhage and cellular debris in the lumens. The bronchial epithelium was swollen, vacuolated and disrupted. Type II pneumocytes were frequently seen in PTFE pyrolysis products treated birds, in contrast to controls, where type II were not seen. There was subepithelial edema with cellular debris and hemorrhage (Figures 20 and 21). Air capillary spaces were severely compressed or obliterated and contained electron-dense membrane remnants and cellular debris (Figures 22 and 23). Erythrocytes were present in large numbers and were enveloped in endothelial membranes or were free in air capillary spaces (Figures 22 and 23). Blood capillary endothelium was generally intact but swollen (Figure 23).

Lung changes were much less severe in birds exposed to sublethal doses of PTFE pyrolysis products. Congestion was evidenced by increased numbers of erythrocytes in blood capillaries, but air capillaries were mostly intact. A few air capillaries had loosened or free membranes in the spaces and some epithelial cells were degenerated (Figure 24). Some 3° bronchi had the same lesions as in the more severely treated birds, but many bronchi were normal. There was no evidence of foreign particulates other than the common stain precipitates in any of the bird groups.

DISCUSSION

The results from these experiments prove that the pyrolysis products of overheated PTFE-lined pans are acutely

toxic to parakeets. This susceptibility of parakeets and other small birds has been reported by others.[3,10] Parakeets can be killed under the same exposure conditions that evoke no clinical signs in human beings and other mammals.[5,16] Pyrolysis products of PTFE contain particulates of respirable size that are inhaled by the birds.[5,12] These particulates are toxic and contain or act as vehicles for other toxic compounds such as hydrogen fluoride, carbonyl fluoride, and perfluoroisobutylene. The fumes are acidic and cause direct damage to the delicate cell membranes of the lung tissue.[4,18] These severe lesions will explain the clinical signs and acute toxicosis in exposed birds. The response of the parakeets to these toxic compounds was measured by close observation of clinical signs and the gross and microscopic tissue changes.

The first clinical sign was eyelid blinking and was most likely caused by direct chemical irritation on contact with the toxic fumes. The eyelid movements could also have been eyelid drooping associated with somnolescence from the hypoxia of pulmonary dysfunction.[14] The panting, gasping, anxiety, and cage wire biting were physiological and behavioral reactions to respiratory difficulties.[14] Normal respiratory movements in the bird should not be obvious and are quiet. Incoordination, seen as rocking and bobbing, and inability to stand were manifestations of the anoxia resulting from the ineffective respiration in the severely damaged lung tissue.[14]

Some knowledge of the avian respiratory system is necessary to visualize and understand the lesions in the affected birds. The avian lung is markedly different from that of mammals. In the most simple terms, the mammalian lung can be described as an elastic container filled with an ever-dividing bronchial tree that has many terminal or dead-end functional units called alveoli. The air flow is to and fro with gas exchange taking place in the thin alveolar walls. In contrast, the avian lung is a fairly rigid fixed-in-place container with a complex system of large airways, with primary (1°), secondary (2°) and tertiary (3°) bronchi (or parabronchi) that interconnect via the small air tubes called air capillaries. The actual functional units of the lung are the numerous air capillaries that surround and open into the 3° bronchi. These narrow thin-walled air tubes are analogous to mammalian alveoli but are not blind sacs. Instead, they are continuous and by anastomosis join one 3° bronchus to another.[14,19,20] Air circulates, rather than moving to and fro, through this network by the bellows action of the 7 to 9 pairs of air sacs found throughout the body.

In this study, the gross lesions of severe pulmonary congestion and hemorrhage were consistent with the histologic changes. On light microscopy, pulmonary edema was not found to be significant, which is contrary to other reports.[3,6,10,21]

The basic microscopic lesions in affected birds were the loss of integrity of 3° bronchial walls and obliteration of air capillaries. The resultant loss of air exchange membranes and the obstruction of 3° bronchi with hemorrhagic exudate

are responsible for the clinical signs of respiratory distress and anoxia. Acute chemical or irritant pneumonitis would be an etiologic diagnosis of the lesions due to these pyrolysis products, but it is not very descriptive. The morphologic diagnosis of severe acute necrotizing and hemorrhagic pneumonitis is descriptive and does indicate the severity of the lesions found in affected birds.

The constriction or spasm of the 3° bronchi, as evidenced by reduced lumen diameters and contracted muscular bands, contributed to the decrease in air flow to any remaining air capillaries.[14] The remarkable dark discoloration of the blood seen on gross examination could be attributed to the retarded pulmonary blood flow and lack of oxygenation.[14]

The significance of the yellow-brown particulates seen on light microscopy is questionable, although others have considered them to be the toxic particulates.[6]

Exposure to PTFE pyrolysis products for 9 minutes or longer was lethal to all but 1 bird and produced severe clinical signs and lesions. Five-minute exposure was less toxic and was not lethal in 24 hours postexposure. As expected, the fumes from heated plain aluminum pans did not produce clinical signs or lesions. Because of this, the birds in Exposure Procedure V were considered to be negative controls and essentially normal for comparison with the other birds. Exposure Procedure IV was designed as a negative control, but PTFE pyrolysis products from previous experiments condensed in the delivery system as a light brown powder. Repyrolysis of this condensate was lethal to the birds in

this group. The problem of condensing pyrolysates was mentioned in the literature but was mistakenly ignored in setting up this exposure.[21] This mistake in experimental design proved that small amounts of pyrolysis products are lethal to parakeets. In man, a mere 0.4 mg of PTFE on a cigarette will produce polymer-fume fever.[22]

These experimental exposures did not duplicate natural exposure conditions. Lethal times and concentration of pyrolysis products could be different. But Ersham has demonstrated that under field conditions overheated PTFE-lined pans will produce toxic substances in concentrations that are lethal to parakeets, parrots, and chicks in less than 1 hour.[10] The 400 C pyrolysis temperature used in the present study was 120 C above the 280 C approximate lethal temperature (ALT) for parakeets as reported by Griffith et al.[3] The 6.5 l/min flow rate, used with all birds except 1 (Bird W), was about 2 times the flow rate used to determine the 280 C ALT. The higher temperature and faster flow rate were used to assure a toxic dose of pyrolysis products in this study. However, the 400 C temperature was not an unreasonable choice even for field conditions. A conventional electric stove will heat an empty PTFE-lined pan to 400 C within 8 minutes.[m] When the pan reaches 400 C, the red hot burner surface is over 600 C and would probably heat the pan to temperatures greater than 400 C.

Normal open pan cooking temperatures are in a range of 130 to 280 C and are usually below that which produces measurable amounts of pyrolysis products.[3,23] Cooking oils

and butter will flame and other foods will smoke and burn at 280 C. The food smoke and fire would alert the cook before significant PTFE pyrolysis products were evolved. But overheated empty PTFE-lined pans or PTFE-lined water pans boiled dry are a different matter and would reach pyrolysis temperature quickly.

In summary, parakeets are acutely susceptible to the toxic pyrolysis products of PTFE-lined cookware. The clinical signs, gross lesions, and microscopic tissue changes are all consistent with severe pulmonary damage and anoxia due to the inhalation of toxic fumes.

Table 1. Summary of experiments and results

Exposure			Birds			Results		
No.	Type	Group Time (min)	No.	Sex	Wt. (g)	Lethal Time (min)*	Clinical Signs	Gross and Histologic Lung Lesions
I	Exposed to PTFE pyrolysis products until all dead	3†	W	F	32	3	Severe	Widespread; severe in 15 of 16; No. 11 less severe
		21	G	M	30	21	Severe	
		27	1	M	29	21	Severe	
			2	F	29	22		
			3	F	37	27		
			4	M	33	22		
			5	F	30	18		
			6	M	31	26		
		18	13	M	28	17	Severe	
			14	F	30	18		
II	Exposed to PTFE pyrolysis products for 9 min	9	7	M	33	10	Severe	
			8	M	33	40		
			9	M	29	60		
			10	F	32	UNK≡	UNK≡	
			11	F	30	Survivorδ	Panting	
			12	F	36	UNK≡	UNK≡	
III	Exposed to PTFE pyrolysis products for 5 min	5	15	M	30	Survivorδ	NONE	Focally or locally severe with areas of normal
			16	M	31			
			17	F	26			
			18	F	26			

Table 1 (continued)

Exposure			Birds			Results		
No.	Type	Group Time (min)	No.	Sex	Wt. (g)	Time (min)*	Clinical Signs	Gross and Histologic Lung Lesions
IV	Exposed to pyrolysis products of aluminum and dirty delivery systems	43	19	M	28	43	Delayed but severe	Severe
			20	F	32	43		
			21	M	30	34		
			22	M	32	37		
			23	M	35	26		
			24	F	30	26		
V	Exposed to pyrolysis products of aluminum and clean delivery systems (control group)	65	25	F	32	Survivorδ	None	None (normal)
			26	M	23			
			27	F	27			
			28	F	34			
			29	F	38			
			30	F	31			

*Time of death in minutes from onset of exposure.

†Exposed at 55.4 1/min flow rate. All other birds exposed at 6.5 1/min.

≡Lethal times and clinical signs not known because birds died unattended 2 to 11 hours after onset of exposure. UNK = unknown.

δIt is assumed that the survivors had no significant clinical signs while unattended.

Figure 1. Exposure apparatus. The exhaust fan (a) evacuates the tank and draws in the fumes from the heated pan (b) via the stainless steel delivery system (c). The fumes are directed through the exposure cage (d) by partial baffle (e). The indicating pyrometer (f) has a thermocouple at the center of the pan.

Figure 2. Schematic of exposure apparatus illustrates air flow.

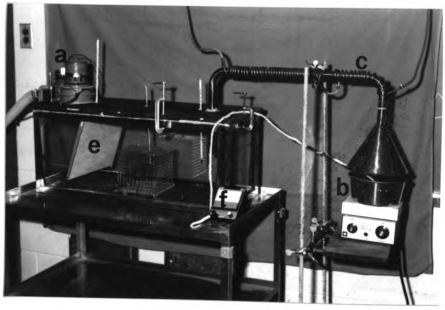

Figure 1

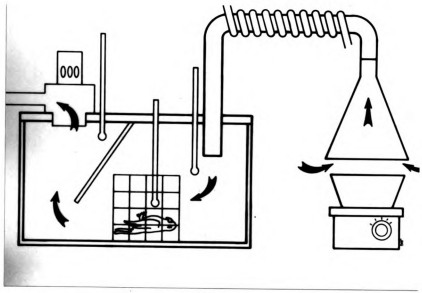

Figure 2

Figure 3. Gross photograph of lungs from a control bird (normal). Note the light color of the tissue and the prominent 3° bronchi (parabronchi) visible on the surface.

Figure 4. Gross photograph of lungs from a bird after lethal exposure to PTFE pyrolysis products. Note the diffuse dark coloration of the tissue from congestion and hemorrhage.

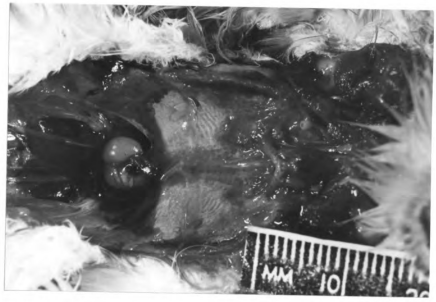

Figure 3

Figure 4

Figure 5. Gross photograph of lungs from a bird after a sublethal exposure to PTFE pyrolysis products. Note the presence of both normal and abnormal areas of coloration. In normal areas 3° bronchi are visible on the surface.

Figure 6. Photomicrograph of normal lung tissue from a control bird (Exposure Procedure V). The prominent longitudinal sections of 3° bronchi are open with many atria seen as sacculations in the bronchial walls. The parenchyma has an open lace-like appearance. H&E; X80.

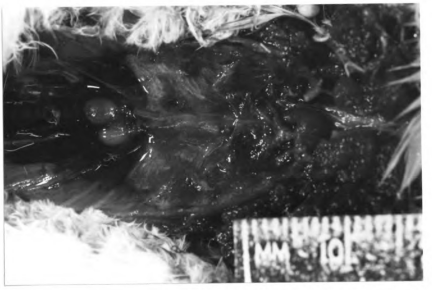

Figure 5

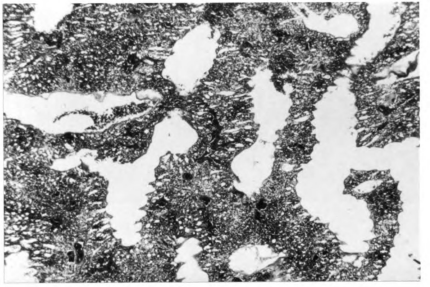

Figure 6

Figure 7. Photomicrograph of normal lung tissue from a control bird (Exposure Procedure V). The center of this field features an open clear cross section of a 3° bronchus. It has severel large atria and is surrounded by many air capillaries. H&E; X200.

Figure 8. Photomicrograph of normal lung tissue from a control bird (Exposure Procedure V). This field centers on a thin 3° bronchial wall with a narrow band of smooth muscle (open arrow) and several atria (closed arrows). The ridges forming the atria have small cross sections of smooth muscle. The lumen is clear of exudate and the air capillaries beneath the bronchial wall are seen as many open spaces surrounded by cells. Toluidine blue, 1 μm section; X525.

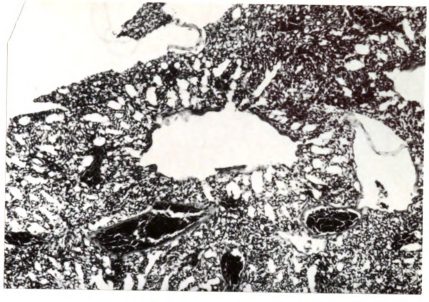

Figure 7

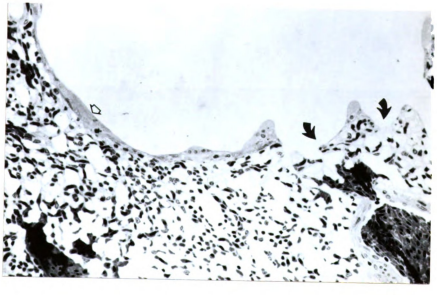

Figure 8

Figure 9. Photomicrograph of normal lung tissue from a control bird (Exposure Procedure V). The flattened epithelium of a 3° bronchus and many thin-walled air capillaries surrounded by blood capillaries are seen. Toluidine blue, 1 μm section; X800.

Figure 10. Photomicrograph of lung tissue from a bird after lethal exposure to PTFE pyrolysis products (representative of Exposure Procedures I, II and IV). Note the 3 prominent longitudinal sections of blood-filled 3° bronchi and the dense congested parenchyma with obliterated air capillaries. H&E; X80.

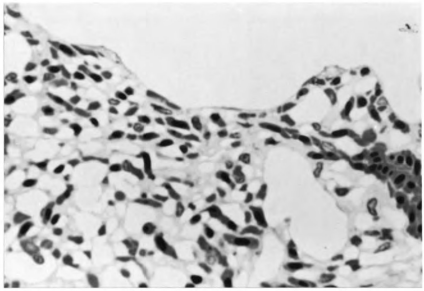

Figure 9

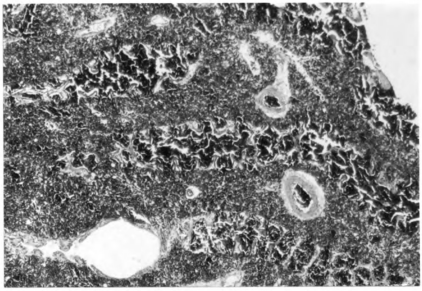

Figure 10

Figure 11. Photomicrograph of lung tissue from a bird after lethal exposure to PTFE pyrolysis products (representative of Exposure Procedures I, II and IV). Note the cross section of a blood-filled 3° bronchus (center) and dense congested parenchyma. Air capillaries are not identifiable. H&E; X200.

Figure 12. Photomicrograph of lung tissue from a bird after lethal exposure to PTFE pyrolysis products (representative of Exposure Procedures I, II and IV). This contracted 3° bronchus has erythrocytes in the lumen (l) and swelling and vacuolation of the epithelium (arrows). Toluidine blue, 1 μm section; X800.

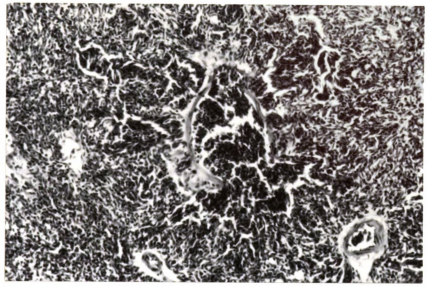

Figure 11

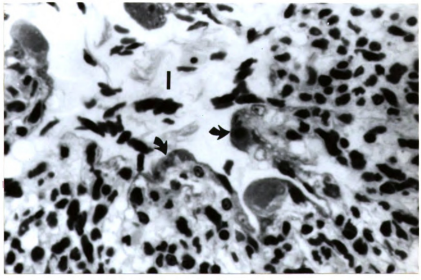

Figure 12

Figure 13. Photomicrograph of lung tissue from a bird after lethal exposure to PTFE pyrolysis products (representative of Exposure Procedures I, II and IV). The blood-filled 3° bronchus is lined with swollen and loosened epithelium (arrows). The parenchyma is congested. Toluidine blue, 1 μm section; X800.

Figure 14. Photomicrograph of lung tissue from a bird after lethal exposure to PTFE pyrolysis products (representative of Exposure Procedures I, II and IV). This 3° bronchial wall is disrupted and erythrocytes can be seen escaping into the lumen (arrow). There is subepithelial edema (e). Toluidine blue, 1 μm section; X2050.

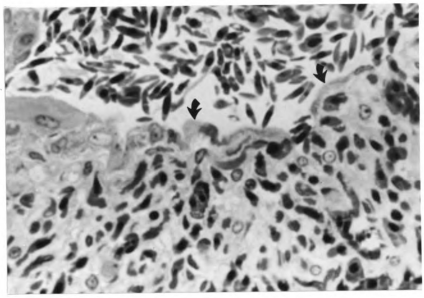

Figure 13

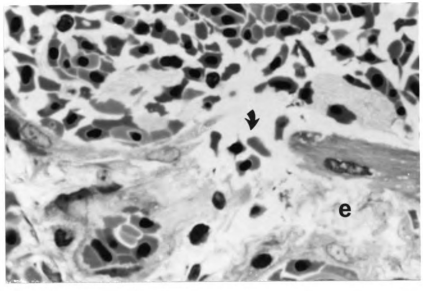

Figure 14

Figure 15. Photomicrograph of lung tissue from bird 11, which survived 24 hours after Exposure Procedure II. There is mucous exudate in this 2° bronchus. H&E; X525.

Figure 16. Photomicrograph of lung tissue from a bird 24 hours after sublethal exposure to PTFE pyrolysis products (Exposure Procedure III). Compare this cross section of a 3° bronchus to those in Figures 6 and 10. H&E; X200.

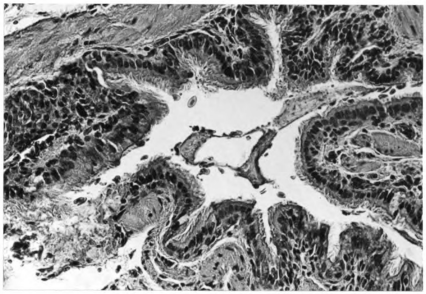

Figure 15

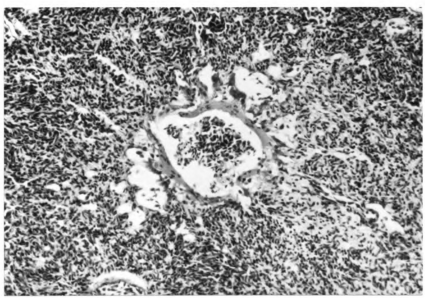

Figure 16

Figure 17. Control Group (Procedure V). Normal lung tissue bordering a 3° bronchus. The bronchial lumen (l) is clear of any material and lined with flattened epithelial cells (ep). The parenchyma is made up of patent air capillaries (ac) and blood capillaries (bc) containing nucleated erythrocytes. TEM, X4300.

Figure 18. Control Group (Procedure V). Normal lung parenchyma with air capillaries of various sizes (ac). Air capillaries are lined with a thin electron-dense membrane (open arrow). An air capillary epithelial cell nucleus (ep), endothelial cell nucleus (en), and venule (v) are present. TEM, X4300.

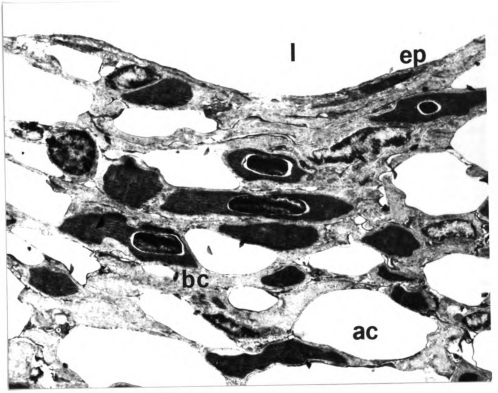

Figure 17

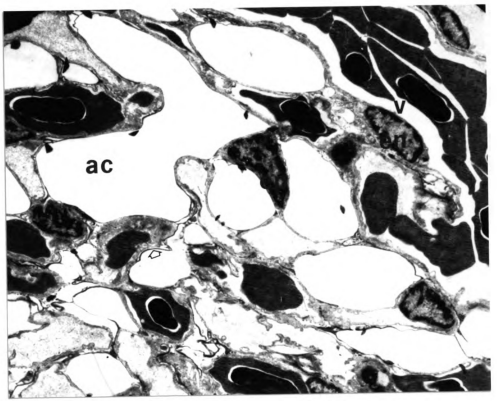

Figure 18

Figure 19. Control Group (Procedure V). Normal lung parenchyma illustrating the envelopment of air capillaries (ac) by blood capillaries (bc). The thicker, less electron-dense endothelial membrane (solid arrows) is closely apposed to the thin, dense air capillary membrane (open arrows). TEM, X4300.

Figure 20. Lethal Dosage Group (Procedure I). Abnormal lung parenchyma bordering a 3° bronchus with blood-filled lumen (l) and swollen, disrupted epithelium (ep). Subepithelial edema is evident. Air capillaries (ac) are seen only as compressed spaces containing membrane remnants (open arrows). Erythrocytes are present in larger numbers than the control. TEM, X4300.

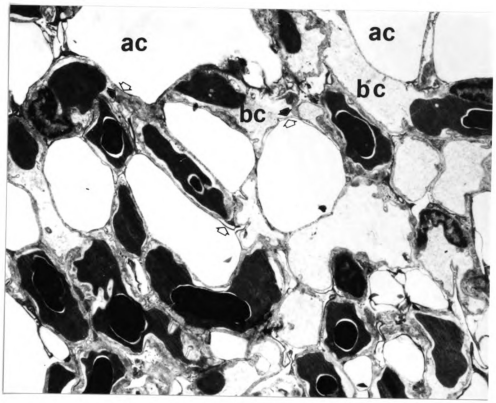

Figure 19

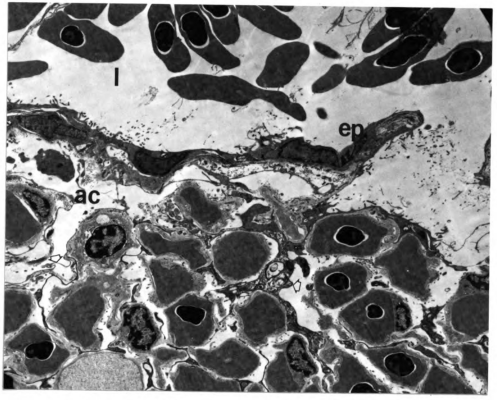

Figure 20

Figure 21. Lethal Dosage Group (Procedure I). Abnormal lung parenchyma with appearance similar to Figure 20. Bronchial lumen (l) is blood filled and the bronchial epithelium is necrotic. Air and blood capillary membranes are disrupted (arrows). TEM, X4300.

Figure 22. Lethal Dosage Group (Procedure I). Abnormal lung parenchyma dense with erythrocytes and disrupted air and blood capillary membranes. TEM, X4300.

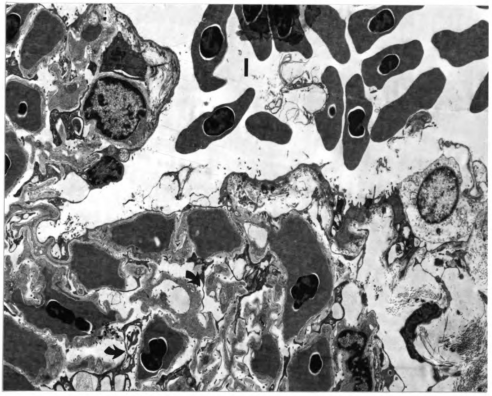

Figure 21

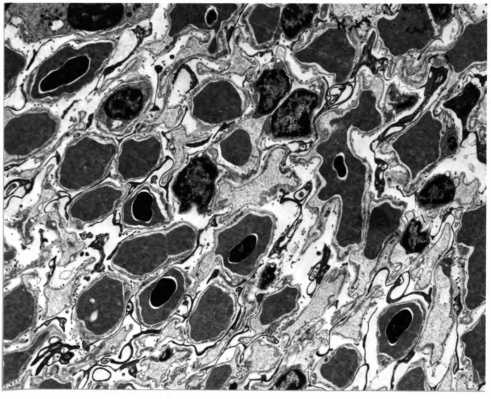

Figure 22

Figure 23. Lethal Dosage Group (Procedure I). Abnormal lung parenchyma similar to Figure 22, but with increased air capillary membrane remnants in the air capillary spaces (open arrows). The erythrocytes appear to be enveloped by endothelium, although normal blood capillaries are not apparent. TEM, X4300.

Figure 24. Sublethal Dosage Group (Procedure III). There are 5 patent air capillaries in this field. One of the air capillary membranes is degenerated and appears to be unfolding or loosening with protrusion into the lumen (open arrow). TEM, X4300.

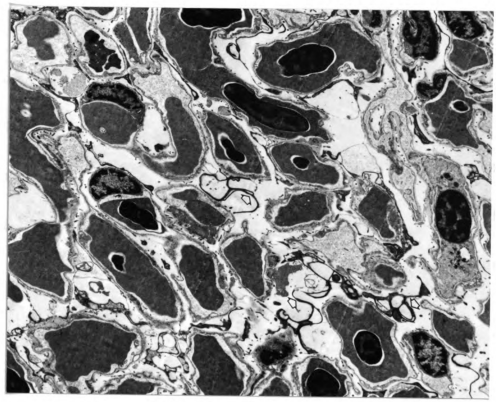

Figure 23

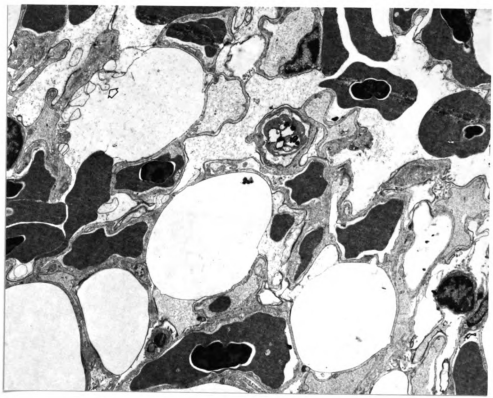

Figure 24

FOOTNOTES

[a]E. I. duPont de Nemours & Co. (Inc.), Wilmington, DE.

[b]Haskell Laboratory for Toxicology and Industrial Medicine, E. I. duPont de Nemours & Co. (Inc.), Wilmington, DE.

[c]Topcrest Cookware, with Teflon II, Topco Associates, Inc., Skokie, IL.

[d]Tek Pro Heatstir 36, American Hospital Supply Corp., Evanston, IL.

[e]Fisherbrand Indicating Pyrometer, Fisher Scientific Co., Pittsburgh, PA.

[f]Topcrest Cookware, Topco Associates, Inc., Skokie, IL.

[g]Dayton Electric Manufacturing Co., Chicago, IL.

[h]Fleisch Pneumotachograph, Dynasciences, Bluebell, PA.

[i]Statham PM-5 transducer, Statham Instrumentation Co., Hato Rey, PR.

[j]Simultrace Recorder VR-6, Electronics for Medicine, Inc., Roxbury, MA.

[k]Mortite Caulking Cord, Mortite Co., Kankakee, IL.

[l]Elektronenscopen EM 9-5-2, Carl Zeiss, Hamburg, Germany.

[m]Personal observation in the kitchen, measured with an indicating pyrometer at the center of the pan.

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ARTICLE 2

A SCANNING ELECTRON MICROSCOPIC STUDY OF POLYTETRAFLUOROETHYLENE PYROLYSIS PRODUCTS TOXICOSIS IN PARAKEETS

INTRODUCTION

Pyrolysis products of polytetrafluoroethylene (PTFE) are known to be toxic to human beings and animals.[1-6] As many as 9 different compounds may be evolved from overheated PTFE, but hydrogen fluoride, carbonyl fluoride, and perfluoroisobutylene are the principal toxic factors.[3,4,7-9] The toxic fraction is in particulate form and has been effectively filtered from PTFE pyrolysate using membranes with 0.2 to 0.45 μm pore sizes.[3,8] In man, occupational exposure to PTFE pyrolysis products causes a transient influenza-like syndrome called polymer-fume fever. Experimental exposures to PTFE pyrolysis products have been lethal to rats, mice, guinea pigs, and other laboratory mammals. In these animal studies, the lower respiratory system appeared to be the target for direct damage from inhaled toxic substances in the pyrolysates. The gross and histologic lesions consisted of pulmonary congestion and hemorrhage which resulted from degeneration and necrosis of alveolar respiratory epithelium. These lesions were typical for chemical pneumonitides caused by the deposition of irritants in the lung parenchyma.[10,11]

Small birds are particularly sensitive to inhaled irritants and noxious gases.[12] For ages canaries have been used as sentinels for detection of toxic air in mine shafts. Parakeets have been found to be more susceptible to PTFE pyrolysis products than other animals tested when exposed to the fumes from overheated PTFE-lined cooking pans.[a,1,13] The gross and histologic lesions are similar to those seen in

the mammalian studies. The microscopic tissue changes have been studied using both light and transmission electron microscopy.[b] There are 2 published accounts of the deaths of pet caged birds shortly after the accidental overheating of PTFE-lined cooking pans.[13,14]

The avian lung differs markedly from mammalian lung and has been the subject of much study.[15-19] The avian lung is a fairly rigid organ made up of a complex system of interconnecting air tubes. In contrast, the mammalian lung is very flexible and consists of arborizing air tubes (bronchi) that terminate at the alveoli. The avian lung circulates air by the pumping or bellows action of the corporal air sacs, whereas the mammalian lung moves air to and fro by expansion and contraction of the lung volume.

In most birds, each lung has a single primary (1°) bronchus that courses caudally along the medial aspect of the organ and exits in the abdominal air sac. Along the way, it gives off several groups of lateral secondary (2°) bronchi (parabronchi). The tertiary (3°) bronchi connect cranial and caudal groups of 2° bronchi. Thick spiral bands of smooth muscle form prominent ridges in the walls of 3° bronchi. The depressions or valleys between the ridges are divided into atria (infundibula) by thinner muscular bands. Each atrium has a number of small ostia that open into the air capillaries. Air capillaries are long narrow tubes that freely anastomose and also connect to other 3° bronchi. The air capillaries are present in large numbers, surrounding the atria and 3° bronchi, and are the principal gas exchange

surfaces of the avian lung. The gas exchange membrane consists of the air capillary epithelium, a basal lamina, and the endothelium of surrounding blood capillaries. The air capillary is analogous to the mammalian alveolus, but instead of being a blind sac it is a continuous and anastomosing tubule. Gas exchange in the mammalian alveolus and the avian air capillary depends on facilitated diffusion from air supplied by to-and-fro movement in the mammal or by a flow-through system in the bird.

The three-dimensional visualization of microscopic anatomy, as seen with a scanning electron microscope, can be helpful in understanding normal avian lung and in the interpretation of changes in pathologic lung. Panghorn et al. (1970) have published a study on the scanning electron microscopic (SEM) anatomy of the avian lung using healthy quail as subjects.[20] Since there are some differences in lung structure between species of birds, the parakeet lung may be quite different from the published details on quail lung.

The purpose of the present study was to use SEM to observe and describe some of the important anatomic features of normal parakeet lung and trachea and to compare these results to those of the tissue from birds exposed to the pyrolysis products of PTFE-lined cooking pans.

MATERIALS AND METHODS

Experimental Procedure

Thirty-two healthy parakeets (*Melopsittacus undulatus*), 4 to 7 months of age, of both sexes, weighing 27 to 38 grams, were exposed to the pyrolysis products from heated (400 C) PTFE-lined and plain aluminum cooking pans at a constant rate for varying periods of time. The exact methods and procedures for these exposures were described in detail elsewhere.[b] There were 5 original exposure procedure groups, but for the purpose of this study the birds were placed into 3 groups: (1) control group - 6 birds were exposed to fumes from heated (400 C) aluminum pans; (2) lethal dosage group - 22 birds were exposed to acutely lethal doses (9 minutes or longer exposure time) of pyrolysis products from heated (400 C) PTFE-lined cooking pans; (3) sublethal dosage group - 4 birds were exposed to sublethal doses (5 minutes exposure time) of pyrolysis products of PTFE-lined pans and survival 24 hours postexposure.

Necropsy Procedure

Necropsies were done on the birds in the lethal dosage group immediately after the exposure period. The birds in the sublethal dosage and control groups were killed by cervical vertebral dislocation and necropsies immediately performed. Whole right lungs and distal tracheas were removed for fixation and processing.

Scanning Electron Microscopy (SEM)

The tissues were fixed in 1:4.5 cacodylate-buffered Karnovsky's solution at 4 C for 24 hours. After the initial fixation, the tissues were trimmed with razor blades in at least 2 planes to expose the various anatomic areas of interest. Trimmed tissues were washed in 0.2 M cacodylate buffer solution and then postfixed in 1:1 cacodylate-buffered 2% osmium tetroxide at 4 C for 8 to 12 hours (overnight). The postfixed tissues were washed in cacodylate buffer and then dehydrated in 10% incremental concentrations of aqueous ethanol solutions with 5-minute immersions in each solution. Two immersions in 100% ethanol were used. The dehydrated tissues were critical point dried using carbon dioxide,[c] mounted on aluminum SEM stubs with adhesive,[d] and then sputter coated with gold.[e] The specimens were subsequently viewed on a scanning electron microscope[f] at 15 Kv. Photographs were made using positive-negative film.[g]

Particulate Collection

A polycarbonate microfilter membrane[h] with 0.2 μm pores was used to collect the particulate pyrolysis products from a heated (400 C) PTFE-lined pan. The 25 mm diameter membrane was mounted in a plastic holder, attached to a low vacuum source, and held over the heated pan for 2.5 minutes. As a control, the fumes from a heated (400 C) plain aluminum pan were filtered in the same manner. The membranes were trimmed, attached to aluminum stubs, sputter coated with gold, and viewed on the scanning electron microscope.

RESULTS

Membrane Filter Collection

The SEM appearance of the polycarbonate membrane used to filter the heated aluminum pan fumes was identical to the SEM appearance of unused filters published by the manufacturer (Figure 1). The membrane used to filter the fumes from the heated PTFE-lined pan had numerous noncrystalline, roughly spherical or elongate masses adhering to the surface. These particles appeared to frequently coalesce, forming elongate amorphous conglomerates. The single masses were 0.1 to 0.3 μm in diameter. The elongated forms had lengths of 0.5 to 1.5 μm (Figure 2).

Trachea and Lung

The surface features of the distal trachea, primary (1°) bronchi, tertiary (3°) bronchi, atria, and air capillaries of birds in the control and lethal and sublethal dosage groups were observed using SEM magnifications of 26X to 16,000X. Secondary (2°) bronchi were not seen or not recognized as such in any of the tissues, although the lungs were trimmed in at least 2 planes.

Control Group

Examination of the distal trachea of control birds revealed an undulating or irregularly folded mucosal surface. Most areas were ciliated and had scattered raised round structures identified as goblet cell secretory vesicles. Erythrocytes were present in the mucosa in some areas and

were considered to be preparation contaminants (Figures 3 and 4). At higher magnification, the cilia were long, slender, and free of clubbing or clumping (Figures 4, 5 and 6). Non-ciliated cells were found scattered among the ciliated cells as single cells or small groups of cells. Some of these had surface accumulations of secretory globules that aided in identifying them as goblet cells (Figure 6). The surface of 1° bronchi was very much similar to that of trachea, except that there were regular longitudinal folds rather than irregular undulations (not illustrated).

At low magnification, lung tissue had an open or spongy appearance with numerous transverse, oblique, and longitudinal sections through 3° bronchi (Figures 7, 8 and 9). The 3° bronchi were 100 to 150 μm in diameter. The depressions or valleys formed in the areas between the thick spiral muscular bands of the 3° bronchial wall were divided into 5 to 8 smaller depressions (atria) by the thin atrial muscular bands (Figures 9 through 12). Atria and atrial subdivisions had multiple openings that communicated with the air capillaries (Figure 10). Air capillaries were 5 to 10 μm in diameter and were surrounded by a blood capillary network (Figures 10, 12, 13, 14 and 15). The surface of 3° bronchial, atrial and air capillaries consisted of monotonous squamous epithelium and had relatively little debris (Figures 12, 14 and 16).

Lethal Dosage Group

The tracheal and 1° bronchial mucosa of birds in the lethal group differed from the control group. In most

specimens, the cilia were clubbed and adhered at the tips (Figures 17 and 18). Some had focal and extensive areas of partial or complete loss of cilia with only severely damaged cilia and "stubble" remaining on the affected cells (Figures 18 and 19). In some specimens the ciliated epithelium was covered with mucus (Figures 20 and 21). The mucus appeared as granular to smooth layers or plaques that were in contact with the tips of cilia (Figures 20 and 22). Amorphous particles with a size range of 0.1 to 0.4 μm were often on the surface of mucous plaques (Figure 22). In places, the mucus was so uniform that the tracheal and 1° bronchial mucosa appeared to be flat and featureless, except for occasional fissures through which intact cilia could be seen (Figure 23). The tenacity of the mucus was evident by its presence after the repeated washes and rinses in processing.

The lung tissues were dense with erythrocytes and lacked the numerous patent 3° bronchi seen in the control lungs (compare Figures 24 and 9). The few identifiable 3° bronchi were partially filled with erythrocytes or had degenerative epithelium (Figures 25 and 26). The epithelium was roughened and fissured with areas of ulceration (Figures 26 and 27). The surface was strewn with particulate debris resembling the particles on the microfilter membrane (Figures 28 and 29). The air capillary and blood capillary beds that surround the 3° bronchi were necrotic and disrupted. In most areas, air capillaries could not be identified because of extensive hemorrhage, fibrin deposition, and cell debris (compare Figures 15 and 30).

Sublethal Dosage Group

The tracheal and 1° bronchial mucosa had similar but less severe changes than the birds in the lethal group. The appearance of lung tissue was variable within individual specimens and from bird to bird. Some areas were comparable to the lungs of control birds, but other areas were as described in the lethal dosage group (Figures 31 and 32; compare Figures 25 and 33; compare Figures 9 and 34).

DISCUSSION

Scanning electron microscopy was used to study the surface morphology of chemical particulate debris and lower respiratory tissues of parakeets at magnifications of 26X to 16,000X. The three-dimensional appearance of the specimens with SEM permitted physical characterization not possible with light or transmission electron microscopy.

The SEM appearance of particulate PTFE pyrolysis products on the surface of the filter membrane was similar in size and outline to that in a published transmission electron micrograph of PTFE pyrolysis products filtered in a similar manner.[8] The 0.1 to 1.5 μm particles are of a size that could be inhaled and deposited in the lung parenchyma.[21] The particles were amorphous or noncrystalline with a generally rounded shape and were present as single masses or as conglomerates. It is believed that the particles in PTFE pyrolysis products are toxic or contain the toxic fractions.[3,4,8] Filtered pyrolysate has been found to be nontoxic to otherwise susceptible laboratory animals.[8]

The SEM appearance of tracheas and lung tissues from birds in the control group was considered to be normal for the parakeet. In another study,[b] these same birds were determined to be clinically normal and had no gross or histologic lesions on necropsy. The published SEM anatomy of normal quail lung has essentially the same appearance as that described for the control parakeets in this paper. Thus, the normal SEM anatomy of the lower respiratory system of parakeets was established for comparison with that of birds having had severe clinical signs and the gross and histologic lesions of toxicosis due to inhalation exposure to the particulate pyrolysis products of overheated PTFE-lined pans.[b]

The SEM appearance of the tissues from birds exposed to lethal doses of PTFE pyrolysis products differed markedly from the normal or control tissues. The heavy mucus secretion, cilial damage, bronchial epithelial necrosis, bronchial hemorrhage, and air capillary destruction were probably due to direct damage from contact with the inhaled toxic pyrolysis products seen as particles on the mucous plaques and on the surface of 3° bronchial epithelium.[10,11]

The tissues from birds exposed to sublethal doses of pyrolysis products had moderate to severe lesions similar to those in the lethal dosage group, but the distribution was not as widespread and normal (functional) areas were seen.

This study has determined that the SEM surface morphology of the parakeet trachea and lung is comparable to that reported for the quail. The surface morphology of trachea and lung in birds exposed to toxic doses of pyrolysis products

from PTFE lined pans is abnormal and consistent with the changes reported on gross and histologic examination of the tissues. The particles of PTFE pyrolysis products were collected and described. Particles of similar size and shape were found on the surfaces of tissues in exposed birds and may represent the etiologic agents of the severe necrotizing and hemorrhagic pneumonitis.

Figure 1. Polycarbonate filter membrane surface free of particulate matter or other debris after filtration of fumes from a heated plain aluminum pan. SEM, X16,000; bar indicates 1.0 µm.

Figure 2. Polycarbonate filter membrane surface after filtration of fumes from a heated PTFE-lined pan. The surface is covered with particulate debris consisting of generally rounded shapes as single masses or in conglomerates. SEM, X16,000; bar indicates 1.0 µm.

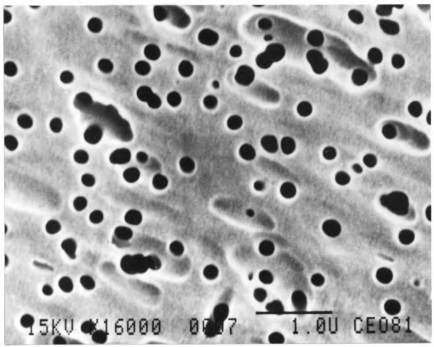

Figure 1

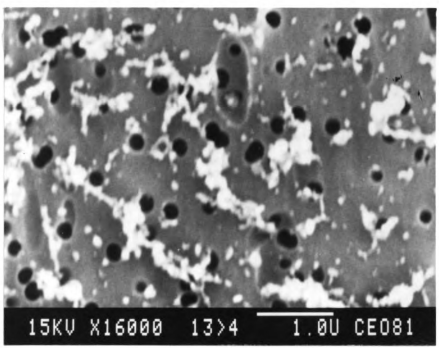

Figure 2

Figure 3. Control Group. Ciliated tracheal mucosa has undulating irregular folds. The elongate oval-shaped structures are contaminant erythrocytes (solid arrow). The raised round structures that appear to be embedded in the mucosa are goblet cell secretory vesicles (open arrow). SEM, X600; bar indicates 10.0 µm.

Figure 4. Control Group. Higher magnification of an area in the center of Figure 3. The mucosal folds, individual cilia, and the goblet cell vesicles are more easily visualized in this view. SEM, X1,500; bar indicates 10.0 µm.

Figure 3

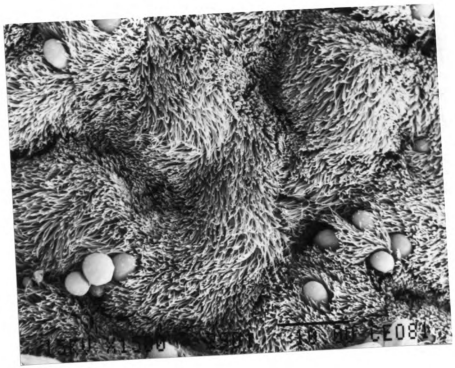

Figure 4

Figure 5. Control Group. The cilia are long, slender, and free of clubbing or clumping. A mucosal gland pore is at center at center right. SEM, X2,000; bar indicates 10.0 μm.

Figure 6. Control Group. The nonciliated goblet cell (lower right) has a surface accumulation of secretory material. The cilia are again seen as long and slender without clumping or clubbing. SEM, X4,400.

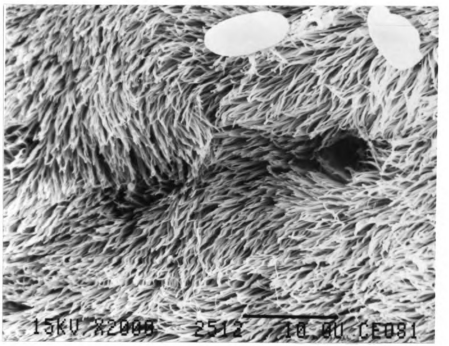

Figure 5

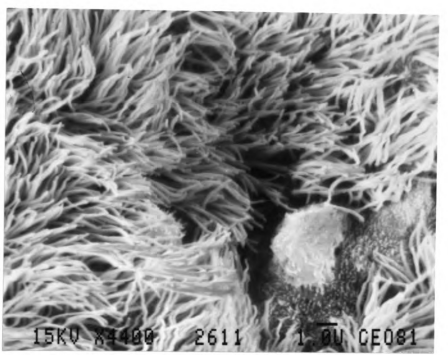

Figure 6

Figure 7. Control Group. At low magnification, the lung has an open or spongy appearance with prominent transverse, oblique, and longitudinal views of patent 3° bronchi. Several large blood vessels are seen (center and center right). SEM, X26; bar indicates 1000.0 µm.

Figure 8. Control Group. Higher magnification of an area in Figure 7. The patency of the 3° bronchi, atria, and air capillaries is evident. SEM, X78; bar indicates 100.0 µm.

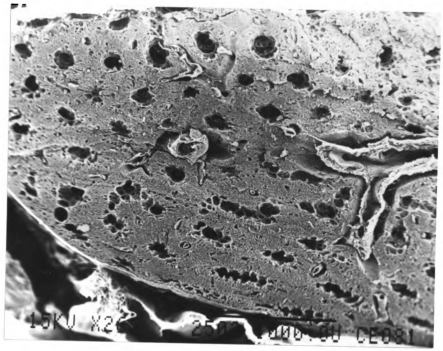

Figure 7

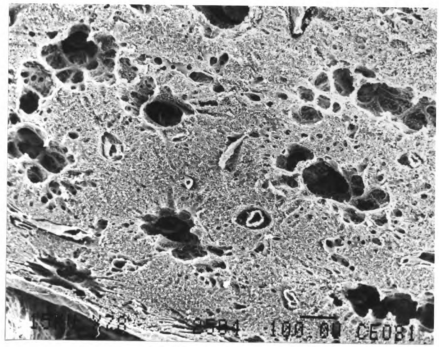

Figure 8

Figure 9. Control Group. This field gives an indication of the number and complexity of the 3° bronchi, seen here as longitudinal sections. The walls of these bronchi are divided into many small compartments. SEM, X60; bar indicates 100 μm.

Figure 10. Control Group. Oblique section of a 3° bronchus. The wall is compartmented by thick spiral muscular bands (A) and thin muscular bands (a). The surrounding parenchyma has many transverse and oblique sections of air capillaries (arrows). Two air capillaries appear to be opening into an atrium (top center arrows). SEM, X260; bar indicates 100.0 μm.

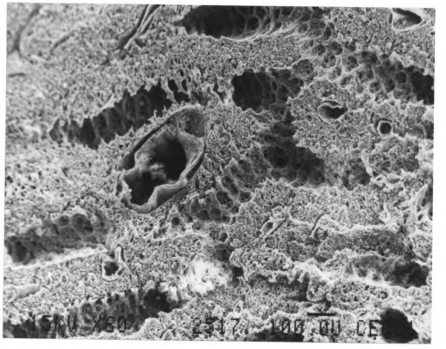

Figure 9

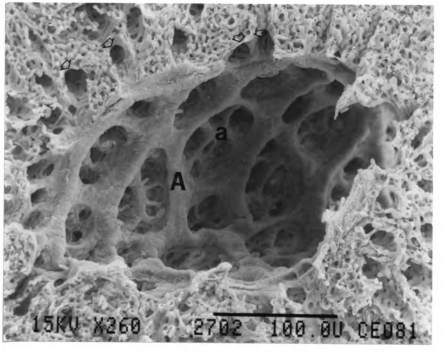

Figure 10

Figure 11. Control Group. Longitudinal section of a 3° bronchus. Cross sections of the spiral muscular bands are seen (lower center). The atrial sacculations or depressions are prominent and several air capillary openings are seen. SEM, X320; bar indicates 100.0 µm.

Figure 12. Control Group. Higher magnification with thick muscular band (at center). Air capillary openings are seen in several atria. SEM, X720; bar indicates 10.0 µm.

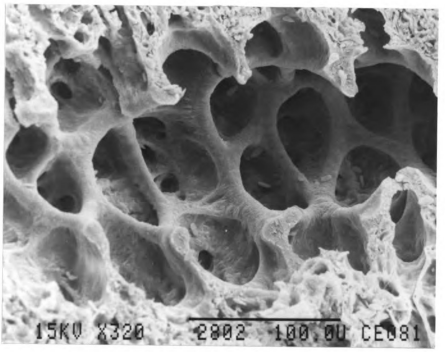

Figure 11

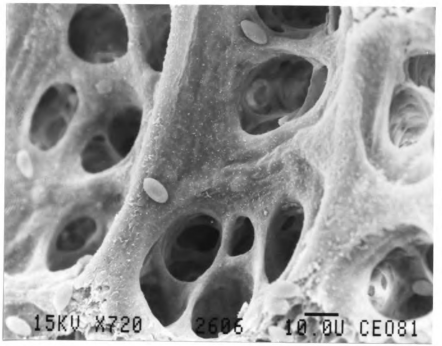

Figure 12

Figure 13. Control Group. Air capillaries (open arrows) are surrounded by a highly vascular tissue. A large blood vessel at an area of branching is seen (large arrow). SEM, X300; bar indicates 100.0 µm.

Figure 14. Control Group. Higher magnification of the tissue in Figure 13. The air capillary lumens range from 5 to 10 µm in diameter. SEM, X860; bar indicates 10.0 µm.

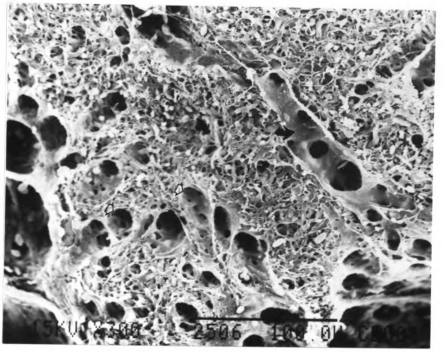

Figure 13

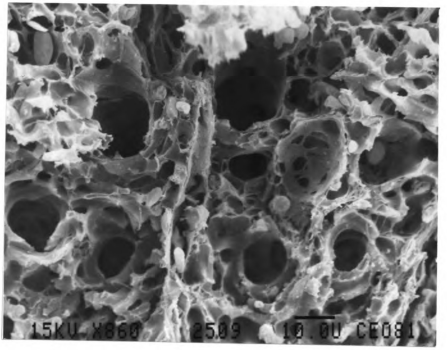

Figure 14

Figure 15. Control Group. Air capillaries are surrounded by blood capillaries (solid arrows). The shapes of erythrocytes can be seen as bulges in the thin air exchange membranes (open arrows).

Figure 16. Control Group. Surface of a 3° bronchial wall has featureless monotonous flattened epithelium and little debris. The fuzzy linear fissures are epithelial cell junctions (open arrow). SEM, X16,000; bar indicates 1.0 μm.

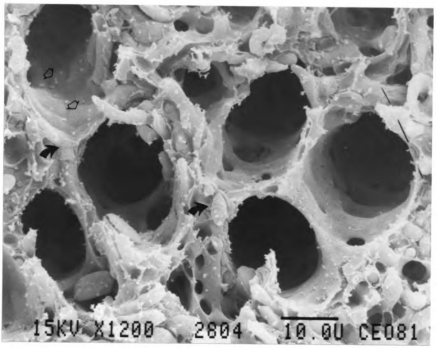

Figure 15

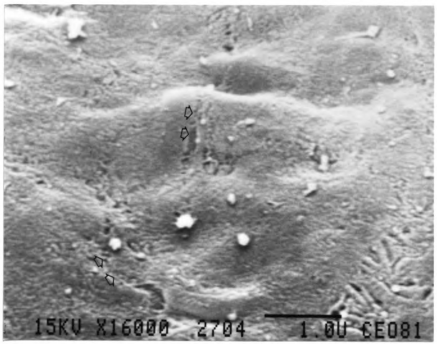

Figure 16

Figure 17. Lethal Dosage Group. The cilia of the 1° bronchial epithelium are clubbed and clumped together. Note the normal longitudinal folds in the mucosa (compared to irregular folds of tracheal mucosa in Figure 3). SEM, X1,200; bar indicates 10.0 µm.

Figure 18. Lethal Dosage Group. An area of severe damage to ciliated epithelium of a 1° bronchus with some injured cilia clinging to the cells. The surrounding less affected cilia have clubbed tips and are adhered. SEM, X2,400; bar indicates 10.0 µm.

Figure 17

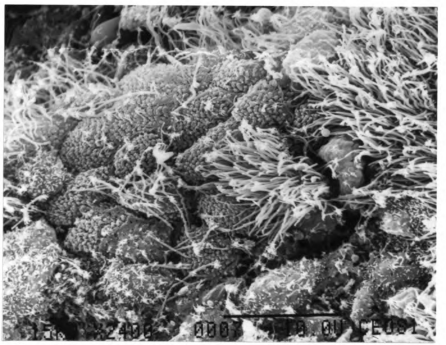

Figure 18

Figure 19. Lethal Dosage Group. An area of severe cilial loss in a trachea with a small patch of less affected cells in the center. Damaged cilia and "stubble" of lost cilia remain. There are wide irregular gaps in some cell junctions suggesting imminent necrosis or sloughing of the cells. SEM, X2,000; bar indicates 10.0 µm.

Figure 20. Lethal Dosage Group. These tracheal cilia are clumped and adhered to mucous plaques. The mucus is granular and stippled with small particles. Several cells have lost cilia and appear necrotic with cracks or fissures in the cell membrane (top center). SEM, X2,000; bar indicates 10.0 µm.

Figure 19

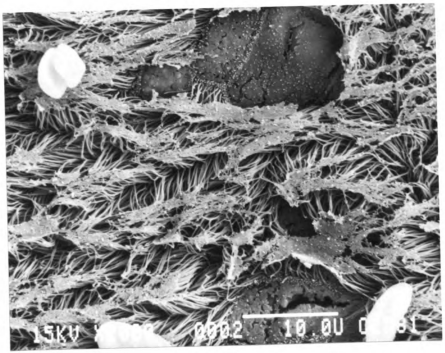

Figure 20

Figure 21. Lethal Dosage Group. The ciliated tracheal epithelium is partially covered with large mucous plaques. Visible cilia are clumped and distorted. SEM, X2,000; bar indicates 10.0 µm.

Figure 22. Lethal Dosage Group. At higher magnification, the particles on the small mucous plaques in Figure 20 are readily seen. The cilia beneath the mucus appear normal. SEM, X9,400; bar indicates 1.0 µm.

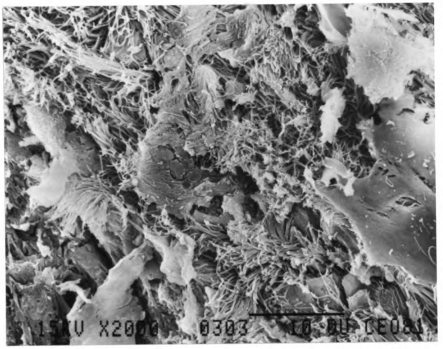

Figure 21

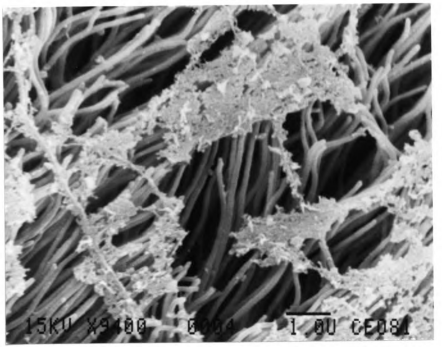

Figure 22

Figure 23. Lethal Dosage Group. Normal appearing cilia are seen through a fissure in a large mucous plaque that covers a large area of tracheal epithelium. SEM, X7,800; bar indicates 1.0 μm.

Figure 24. Lethal Dosage Group. Lung tissue is dense with erythrocytes. Only a few scattered air capillaries are patent and identifiable. The tissue lacks the numerous 3° bronchi seen in Figure 9. SEM, X180; bar indicates 100.0 μm.

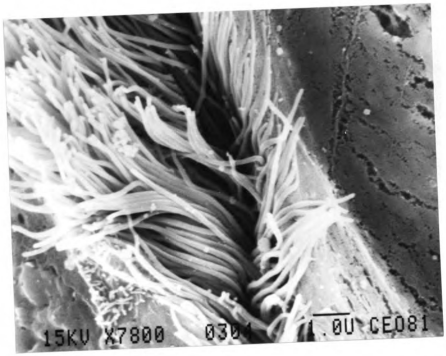

Figure 23

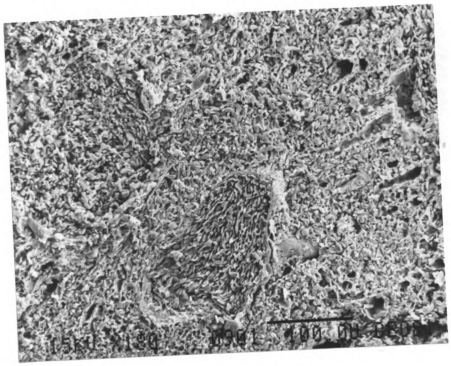

Figure 24

Figure 25. Lethal Dosage Group. The 3° bronchus is partially filled with erythrocytes. Atria and air capillaries are not visible. SEM, X200; bar indicates 100.0 µm.

Figure 26. Lethal Dosage Group. The 3° bronchial epithelium is cracked and roughened. Some areas are ulcerated. SEM, X320; bar indicates 100.0 µm.

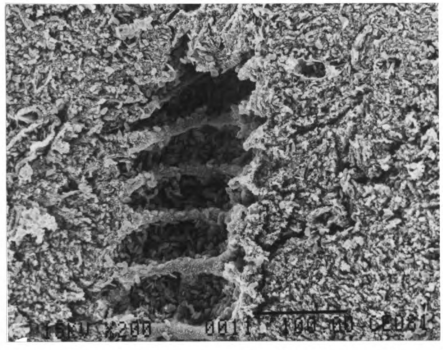

Figure 25

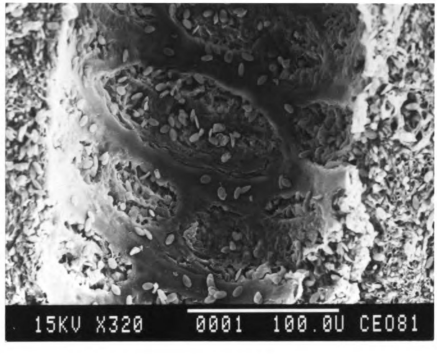

Figure 26

Figure 27. Lethal Dosage Group. Surface of a 3° bronchus is roughened and cracked. SEM, X400; bar indicates 10.0 µm.

Figure 28. Lethal Dosage Group. The surface of a 3° bronchus strewn with particulate debris. An erythrocyte is at lower right. SEM, X2,000; bar indicates 10.0 µm.

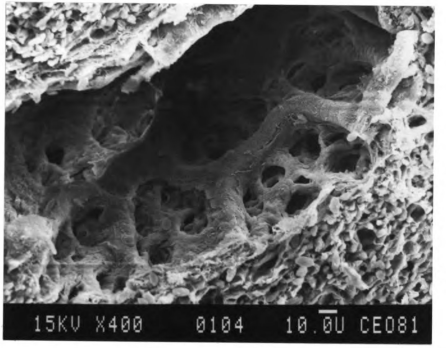

Figure 27

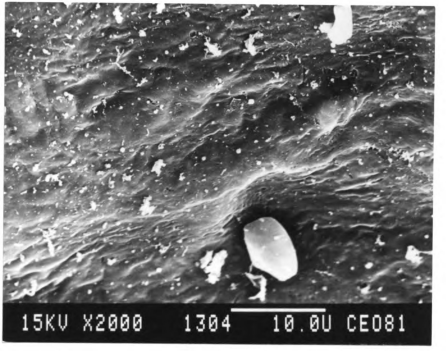

Figure 28

Figure 29. Lethal Dosage Group. At higher magnification, the particles resemble those collected from the heated PTFE-lined pan. SEM, X16,000; bar indicates 1.0 µm.

Figure 30. Lethal Dosage Group. Air capillaries and blood capillaries are disrupted. The tissue is necrotic with fibrin strands, erythrocytes, and cell membrane remnants seen in this field. One partially collapsed air capillary is present (open arrow). SEM, X1,300; bar indicates 10.0 µm.

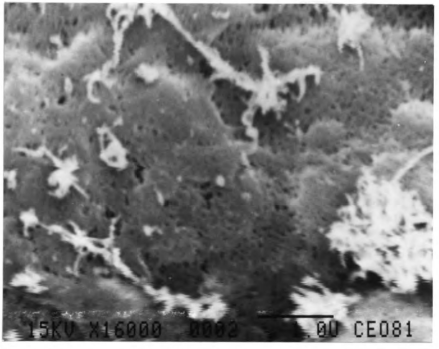

Figure 29

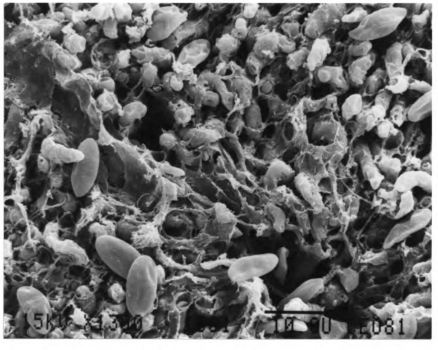

Figure 30

Figure 31. Sublethal Dosage Group. Numerous patent 3° bronchi are present but the parenchyma is congested or hemorrhagic. SEM, X72; bar indicates 100.0 μm.

Figure 32. Sublethal Dosage Group. This lung section, in contrast to that in Figure 31, has blood-filled 3° bronchi. SEM, X100; bar indicates 100.0 μm.

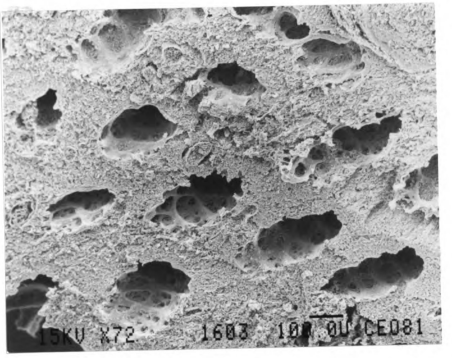

Figure 31

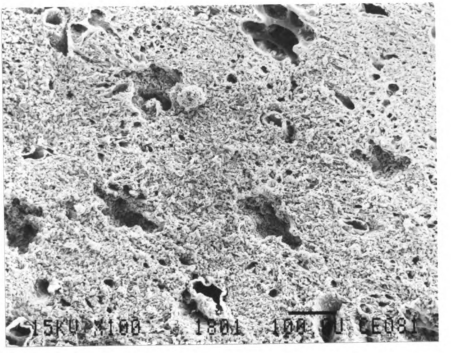

Figure 32

Figure 33. Sublethal Dosage Group. Section of a 3° bronchus partially filled with erythrocytes. SEM, X320; bar indicates 100.0 µm.

Figure 34. Sublethal Dosage Group. In contrast to the bronchus in Figure 33, longitudinal sections of these 3° bronchi are relatively clear of erythrocytes. The atria and atrial subdivisions are particularly well detailed. SEM, X120; bar indicates 100.0 µm.

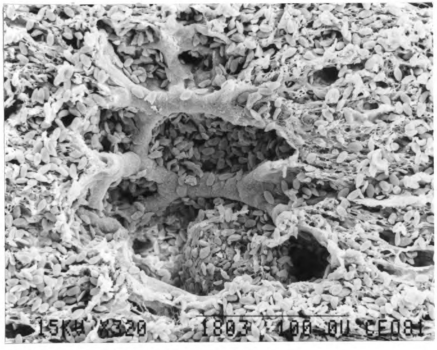

Figure 33

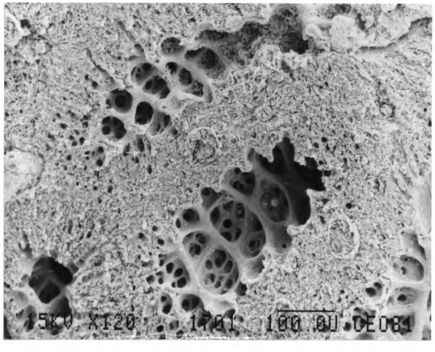

Figure 34

FOOTNOTES

[a]"Teflon" and "Silverstone" tradenames, E. I. duPont de Nemours and Co. (Inc.), Wilmington, DE.

[b]R. E. Wells, M.S. Thesis, Article 1, 1981.

[c]Sorvall Critical Point Drying System, Sorvall, Newtown, CT.

[d]Television Tube Koat, G. C. Electronics Division, Hydrometals, Inc., Rockford, IL.

[e]Mini-Coater, Fill-Vac, Inc., Englewood, NY.

[f]JSM35, Jeal, Tokyo, Japan.

[g]Polaroid Type 665, Polaroid Corp., Cambridge, MA.

[h]Uni-Pore Filters, Bio-Rad Laboratories, Richmond, CA.

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VITA

VITA

The author was born in Dayton, Ohio, on December 1, 1946. He graduated from Fairmont East High School in Kettering, Ohio, in 1965. His parents, James and Betty Wells, still reside in Kettering, Ohio.

The author attended Anderson College, Anderson, Indiana, from 1965 to 1967. In 1967-68 he finished preveterinary undergraduate training at Ohio State University College of Agriculture. He completed four years of professional schooling at the Ohio State University College of Veterinary Medicine and received the degree of Doctor of Veterinary Medicine in 1972.

The author served as a Captain in the U.S. Army Veterinary Corps for two years from 1972 to 1974. He conducted research in food microbiology at the U.S. Army Natick Laboratories, Natick, Massachusetts.

After Army service, the author became a full partner in a large volume mixed practice at the Waynesville Animal Clinic, Waynesville, Ohio. In 1978, he sold his half of the practice and was chief of staff at the Dayton Emergency Veterinary Clinic for five months until moving to Michigan to begin a three-year residency and graduate study program in veterinary pathology at Michigan State University.

The author has completed three years of veterinary pathology residency training at MSU with studies in both anatomic and clinical pathology.

Roger Earl Wells is married to the former Earldean Frances Harmon and is the proud father of two children, Scott Erik and Amanda Erin.