PULMONARY FUNCTION EVALUATION OF CATS AFTER ONE YEAR OF EXPOSURE TO DIESEL EXHAUST

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ABSTRACT

Adult male, inbred, disease-free cats of uniform age and size were exposed eight hours per day, seven days per week to a 1:18 dilution of diesel exhaust emissions. After one year of exposure, the animals were removed from the chambers for measurement of lung volumes, forced expiratory flow rates, dynamic compliance and resistance, diffusing capacity, and nitrogen washout. No important changes in pulmonary function were detected with the exception of a decrease in closing volume ($P < .05$). The inability to detect decrements in pulmonary function may have been due to insufficient concentration of exhaust, insufficient exposure length, or to the use of a species resistant to diesel exhaust.

To test these possibilities, the cats are being exposed for an additional year, and another species, hamsters, are being exposed for future testing at exhaust dilutions of 1:18 and 1:9.

INTRODUCTION

The use of diesel engines has increased greatly in the past 30 years. Much of this increase has been due to a changeover to use of diesel engines in locomotives, heavy trucks, construction equipment, and farm tractors. With increasing fuel costs, the percentage of diesel engine equipped automobiles is also increasing and is expected to continue to increase in the foreseeable future.

Diesel exhaust contains a wide variety of pollutants, many of which are known to cause lung damage (4). Moreover, many of these pollutants are adsorbed on the very fine carbon particulate allowing for effective penetration into the deep lung (8). For these reasons, it was considered important to study the effects of chronic inhalation of diesel exhaust emissions upon lung function.

The present experiment was designed to evaluate the effects of chronic diesel exhaust inhalation upon pulmonary function and pathology in cats.

METHODS

Young adult male cats, born and maintained in a disease free environment and inbred for several generations were purchased from Liberty Labs, Liberty Corners, New Jersey. The cats were uniform in size and varied in age by less than two weeks. The cats were exposed in chambers 1.38 meters square with an interior volume of 2.83 cu m. Each chamber contained a wire mesh floor and two half shelves of similar material at 2/3 and 1 1/3 meters above the floor. This allowed the cats about 4.7 m^2 (50 sq. ft.) of horizontal space. Eight or nine cats were housed per chamber. They were not caged but allowed to roam free. Food and water was provided ad libitum. The cats were exposed eight hours per day, seven days per week for one year to diesel exhaust diluted to produce a particulate concentration of 6 mg/m^3. Details of the exposure conditions have been presented elsewhere in the proceedings by Hinners et al.

Prior to pulmonary function testing, the animals were fasted for one day. The testing followed 18-20 hours of no diesel exposure. The animals to be tested were anesthetized with Ketaset Plus (Ketamine 100 mg, Promazine 7.5 mg per ml) at a dose of 42 mg/kg. Following the induction of anesthesia, an esophageal balloon was placed in the lower third of the esophagus and an 18-22F endotracheal tube was inserted into the trachea with the aid of a laryngoscope. The cuff of the endotracheal tube was inflated and excessive length of the distal end trimmed even with the end of the mouth. The animal was then placed into the chamber, ventral side up, for compliance and resistance testing. For all other tests, the animal was in the prone position, dorsal side up.

Pulmonary mechanics were obtained from simultaneous volume, flow, and transpulmonary pressure tracings displayed on a twelve-channel photographic recorder (Electronics for Medicine, DR-12). Airflow through the pneumotachograph was measured with a differential transducer and electrically integrated to produce a volume trace. Dynamic pulmonary compliance (CL_{DYN}) was calculated from simultaneous volume and transpulmonary pressure tracings at points of zero flow (5). Average flow resistance ($RL_{Ave.\ Flow}$) was calculated from the change in transpulmonary pressure (at equal volumes) divided by the sum of inspiratory and expiratory flow.

All mechanics were measured while the animal was breathing spontaneously through the pneumotachograph only. The animal was inflated for ten seconds initially and periodically throughout the testing to expand atelectatic areas.

The pulmonary function tests requiring breathing maneuvers lung volumes, maximum expiratory flow-volume curve (FEF), diffusing capacity ($DL_{C^{18}O}$), nitrogen washout (ΔN_2), and closing volume (CV) were performed using a variable pressure plethysmographic chamber. The basic method employed was similar to that used in an external tank respirator; however, a hydraulic control system enabled the operator to bring about inspiration, expiration, breath holding, and breathing rate within the anatomical and physiological limits of the animal. Both flow and volume were controlled secondarily by changes in the pressure surrounding the animal. Inspiratory and expiratory airflow could be controlled from very low rates to the maximum within each subject. Likewise, volume could be controlled for both maximum inspiration and expiration. Inspiratory capacity (IC) was obtained by rapid depressurization to -70 cmH_2O from testing tidal position. Prior to the flow-volume testing it was determined that plethysmograph pressures of +70 cmH_2O would be used to produce maximal expirations. Inspection of flow-volume curves at increasing driving pressures showed that flow limitation characteristics had been reached at volumes above 50% Total Lung Capacity (TLC) when the plethysmograph pressure was greater than 70 cmH_2O. Therefore, FEF at 50% and 40% of TLC are values take at an effort-independent zone of the flow-volume curve. The curves were highly reproducible in each animal and demonstrated a low coefficient of variation (2-2.5%) in the effortindependent zone.

To ensure that sufficient intrathoracic driving pressure was developed, esophageal pressure was recorded during forced expirations. A trans-chest-wall pressure gradient was observed; however, intrapleural pressures of 30-35 cmH_2O were achieved which are efficient to produce flow maxima. A volume error, as a result of thoracic gas compression, was calculated to be approximately 3% at 50% TLC with the intrapleural pressure of 30-35 cmH_2O. This error was considered to be irrelevant because the results were compared in animals tested at the same driving pressures.

Breathing manipulations could be performed in anesthetized animals because of the apnea produced on inflation as a result of the inflation reflex documented by Hering and Breuer (3). The inspiratory inhibition had been demonstrated by recording action potentials from the phrenic nerve.

IC and forced vital capacity (FVC) were recorded during a maximum inspiration, followed by a maximum expiration. Flow and volume tracings were recorded, which provided the essential data points for calculating forced expiratory flows and volumes ($FEV_{0.5,1.0}$) and peak expiratory flow (PF). This procedure of maximum inspiration followed by maximum expiration was performed initially and thereafter for all test maneuvers, insuring equal volume and flow histories.

The methods of Brashear et al (1) and Mitchell et al (6), were combined to obtain values for $DL_{C^{18}O}$ and TLC. The calculations for DLCO were performed according to the method described by Wagner et al (9), for $C^{18}O$. Gas analyses were done using a respiratory mass spectrometer (Perkin-Elmer MGA1100).

Distribution was studied using the single-breath nitrogen washout and closing volume according to the methods described by Buist and Ross (2).

RESULTS

The pulmonary function results for exposed and control cats are summarized in Tables 1 and 2. No significant differences were found in mechanical properties, diffusing capacity, uniformity of distribution, or ventilatory performance. Closing volume (volume of Phase 4) was found to differ from the control values; however, this change cannot be interpreted as impairment. The exposed cats demonstrated a lower closing volume than the controls indicating improved function of small airways. This effect is believed to be a Type 2 statistical error or some unexplainable adaptation phenomona. The latter explanation is unlikely based on our experience. It is probably due to high animal to animal variation seen in some of the pulmonary function tests.

A preliminary study was conducted by the EPA in which several animal species were exposed for 20 hours per day for up to two months to a similar concentration of exhaust as used in the

Table 1

Lung Volumes and Forced Expiratory Flow Rates in Cats Exposed to Diesel Exhaust

	EXPOSED N	EXPOSED MEAN	EXPOSED S.D.	CONTROL N	CONTROL MEAN	CONTROL S.D.
LUNG VOLUMES						
IC (ML)	21	278.7	44.81	21	301.9	49.56
FRC (ML)	21	158.2	35.61	21	265.4	42.22
ERV (ML)	21	69.2	24.58	21	67.0	19.05
RV (ML)	21	86.1	36.99	21	104.1	37.67
TLC (ML)	21	415.3	56.02	21	449.5	74.49
RV/TLC (%)	21	20.26	6.936	21	22.72	5.896
DYNAMIC LUNG VOLUMES						
FVC (ML)	21	348.4	43.46	21	368.9	42.14
FEV 5/FVC (%)	21	84.3	8.44	21	81.6	6.39
FEV1/FVC (%)	21	97.6	1.96	21	97.3	1.74
PEFR (ML/5)	21	1016.5	185.10	21	1041.8	174.17
FEF 50% (ML/5)	21	728.0	195.62	21	661.1	160.43
FEF 25% (ML/5)	21	490.4	186.81	20	481.4	199.49
FEF 10% (ML/5)	19	196.5	107.35	20	222.2	156.82
FEF 50%/FVC (FVC/SEC)	21	2.101	.5434	21	2.072	.4249
FEF 25%/FVC (FVC/SEC)	21	1.400	.4806	20	1.314	.5310
FEF 10%/FVC (FVC/SEC)	19	.569	.3131	20	.605	.4294
FEF 40% TLC (ML/5)	21	486.3	252.64	21	557.2	248.05
FEF 40% TLC/TLC (TLC/SEC)	21	1.185	.6355	21	1.317	.6368

Table 2

Dynamic Compliance and Resistance, Diffusion Closing Volume and Nitrogen Washout in Cats Exposed to Diesel Exhaust

	Exposed			Control		
	N	Mean	S.D.	N.	Mean	S.D.
MECHANICS						
(CMH_2O/L/5)	21	10.675	4.5785	21	10.323	4.4305
(ML/CMH_2O)	21	23.536	7.2045	21	23.698	9.2548
DIFFUSION						
DLCO (ML STPD/ MIN/MM HG)	21	1.179	.3143	21	1.217	.3023
DLCO/VA (1/MIN/MM HG)	21	.00345	.00125	21	.00335	.00084
CLOSING VOLUME AND WASHOUT WITH N_2						
CV	21	25.6*	13.44	21	36.8	16.00
CV/VC (%)	21	7.91	3.276	21	10.46	4.6
(CV+RV)/TLC(%)	21	26.83**	6.993	21	20.05	7.192
%N_2/25%VC	21	.32	.206	21	.29	.302

* Significantly different from Controls $P<.05$

** Significantly different from Controls $P<.01$

present study (8). This initial study was designed to provide preliminary data on tolerance levels, toxic effects, and target organs. Some of the findings included detection of the presence of black granular particles in the alveolar macrophages, black pigment in the bronchial and carinal lymph nodes, increased pulmonary flow resistance, increased lung weight/body weight ratios, and sinus bradycardia in guinea pigs.

In the present study, in which animals were exposed for a longer period of time (one year), but only eight hours per day, convincing evidence is present that inhalation of diesel exhaust under these conditions does not result in functional alterations in the lungs of cats. This is in contrast to the preliminary study in which some positive responses were noted. We believe cats in the exposed groups have functionally adapted to this exposure. Increasing the concentrations of diesel exhaust or increasing the duration of this study may produce significant chronic pulmonary disease; however, the present results cannot provide a guide to those parameters which may be early indicators of functional alterations.

Minimal or no responses would be expected to result from the individual gaseous pollutants at the concentrations present. The particulate level might be expected to produce impaired ventilation as a result of the site of deposition and potential tissue response. The effect of the combination of gaseous pollutants, interaction with, or adsorption to the particulate, however, cannot be predicted.

The results reported here are from an ongoing study in which cats are being exposed for a planned two years to diesel exhaust emissions. Shortly after the start of the second year of exposure, the dilution of exhaust was decreased to produce a particulate concentration of 12 mg/m^3. Pulmonary function, pathology, and biochemical parameter will be assessed following completion of exposure. In addition, lung pathology is being evaluated from several cats that died during the year either from unrelated causes or during testing.

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APPENDIX

(Abbreviations)

IC	Inspiratory Capacity
FRC	Functional Reserve Capacity
ERV	Expiratory Reserve Volume
RV	Residual Volume
TLC	Total Lung Capacity
FVC	Forced Vital Capacity
PEFR	Peak Expiratory Flow Rate
FEF	Forced Expiratory Flow
RL	Resistance
Cl	Compliance
L.F.	Low Frequency
H.F.	High Frequency
DLCO	Diffusing Capacity
VA	Alveolar Volume

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