Pulmonary Function Responses in Cats Following Long-Term Exposure to Diesel Exhaust

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Long-term inhalation studies were carried out to evaluate the toxic pulmonary effects of diesel engine emissions. Cats were exposed for over 2 years to whole, diluted diesel exhaust at levels expected to produce frank toxic effects. During the first 61 weeks of exposure, the cats received exhaust having a particulate level of 6 mg m^{-3}. This was followed by a doubling of the exposure level from weeks 62 to 124 resulting in particulate levels of 12 mg m^{-3}. No definitive pattern of pulmonary function response was observed following 61 weeks; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes and diffusing capacity were indicative of a pulmonary interstitial response which was later verified by histopathology.

INTRODUCTION

The advantages of diesel power are many-fold. However, the prime motivation for the use of diesels relates to the reduced cost of operation such as longer engine life, decreased maintenance, cheaper fuel, increased specific energy and increased safety. The increase in safety is related to diesel fuel's lower volatility and flash point with a concomitantly reduced explosion hazard when compared with gasoline. The advantages along with the apparent increased use of diesels and the lack of a well-defined pattern of toxic pulmonary response provided the impetus for this investigation.

The purpose of this study was to identify physiological responses in the lungs of animals exposed to diesel exhaust at sufficiently high concentrations and over a long enough time period to produce a distinct functional pattern of response.

Evaluation of pulmonary function responses to experimentally controlled diesel exhaust is primarily confined to investigations carried out in the past few years. Previous studies are largely epidemiologic and while they are important guides to the potential toxic effects, they lack qualitative and quantitative characterization, essential for analytical dose-response toxicology.

Summarization of previous work involving animal exposures to diesel exhaust and pulmonary function testing includes numerous acute-subchronic and a few chronic studies. Included in the acute-subchronic work are the studies by Wiester,[1] O'Neil[2] and Vinegar.[3] All of these studies were conducted at the Environmental Protection Agency's labs, and therefore possess the same exposure conditions as defined in Table 1.

Briefly, Wiester[1] found increased flow resistance in guinea-pigs exposed for 4 weeks. O'Neil[2] found no significant changes in lung volume or diffusing capacities in mice exposed for 3 months and Vinegar found reduced total lung capacity, vital capacity, residual volume and diffusing capacity in Chinese hamsters following 6 months exposure. Vinegar[3] repeated these tests after a higher exposure during the second year and found greater reductions in the same parameters, possibly indicating a concentration-response relationship.

Long-term or chronic studies include those conducted at General Motors Research Laboratories, reported by Gross;[4] those of Fraunhofer Institute reported by Heinrich *et al.*;[5] the Inhalation Toxicology Research Institute studies reported by Mauderly *et al.*,[6] and EPAs studies reported by Pepelko.[7]

The reported pulmonary function studies by Gross[4] involve inhalation exposure to whole diluted exhaust at particulate concentrations of 1500 $\mu g\ m^{-3}$ of diesel particulate for 20 hours a day, $5\frac{1}{2}$ days a week for 612 days. He presented data showing a trend in volume-normalized data indicating increased functional residual capacity, residual volume, forced expiratory volume in 0.1 s and forced expiratory flows at 40% and 20% of vital capacity in the diesel-exposed rats. While he did not specifically address group mean comparisons at the last test period, total lung capacity, inspiratory capacity, and diffusing capacities showed lower group means in the diesel-exposed rats.

Heinrich *et al.*[5] reported the results of a 24-month diesel exhaust exposure in hamsters and female Wistar rats.[5] The unique feature of these studies related to exposures involving both total exhaust and exhaust without the particulate component. A seven-to-one dilution (air: exhaust) was used with 3.9 mg m^{-3} diesel particulate level in the whole exhaust treatment. Only the rats underwent pulmonary physiological testing and they found no changes in respiratory rate, minute volume, compliance or resistance as measured plethysmographically.

Mauderly *et al.*[6] have reported on interim findings of a life-span study of rodents inhaling whole disel exhaust.

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Table 1. Exposure chamber component concentrations, study averages

		Clean air chamber	Exhaust chambers	
	Units	Weeks 1-124	Weeks 1-61	Weeks 62-124
Dilution factor (air: diesel)		–	18.16 ± 1.72[a]	9.37 ± 1.13
Particulate mass	mg m^{-3}	0.00	6.34 ± 0.81	11.70 ± 0.99
Nitrogen oxides:				
Nitric oxide	ppm	0.05 ± 0.04	11.64 ± 2.34	19.49 ± 3.80
Nitrogen dioxide	ppm	0.03 ± 0.03	2.68 ± 0.80	4.37 ± 1.19
Sulfur dioxide	ppm	0.03 ± 0.02	2.12 ± 0.58	5.03 ± 1.03
Total hydrocarbons (cold)	ppm	2.82 ± 0.50	7.93 ± 1.42	11.02 ± 1.04
Carbon monoxide	ppm	2.20 ± 0.50	20.17 ± 3.01	33.02 ± 2.94
Carbon dioxide	%	0.04 ± 0.002	0.30 ± 0.04	0.52 ± 0.04

[a] Standard deviation of weekly means.

Three exposure levels, 7000, 3500 and 350 $\mu g\ m^{-3}$ of exhaust particulate for 7 h per day, 5 days a week were studied through 30 months. Lung function changes in the Fischer 344 rats at the high and medium levels showed decreased total lung capacities, diffusing capacities and compliance with increased alveolar nitrogen slopes.

EXPERIMENTAL

Diesel exhaust was generated by a Nissan CN6-33 engine coupled to a Chrysler Torque-Flite automatic transmission, Model A 727 (Fig. 1). The engine was cycled continuously at varying speeds in a pattern corresponding to a modified 'California Cycle'. Exhaust, after passing through a muffler, was diluted with filtered and conditioned air, then admitted into the exposure chambers at a rate sufficient to produce 15 volume changes of air per hour. Details of engine exhaust generation, exposure chambers and analytical methods for characterization of atmospheres in the animal exposure chambers have been published elsewhere.[8] A summary of exposure is provided in Table 1. Greater detail of exposure values and characterization was reported by Pepelko.[7] Number two diesel fuel consisting of 64% saturates, 3.4% olefins, 29.9% aromatics and 0.15% sulfur was used.

Twenty-five adult male disease-free cats of uniform age and genetic background were exposed to diesel exhaust. Twenty male cats served as controls. All cats were 13 months old and obtained from Liberty Laboratories where they were maintained in a disease-free environment and inbred for several generations. They were of uniform size (3.63 ± 0.46 kg) and within 2 weeks of the same age. The animals were housed (eight or nine to a chamber) and were allowed to roam free in the chambers. During the first 61 weeks of exposure, the exhaust was diluted to produce a

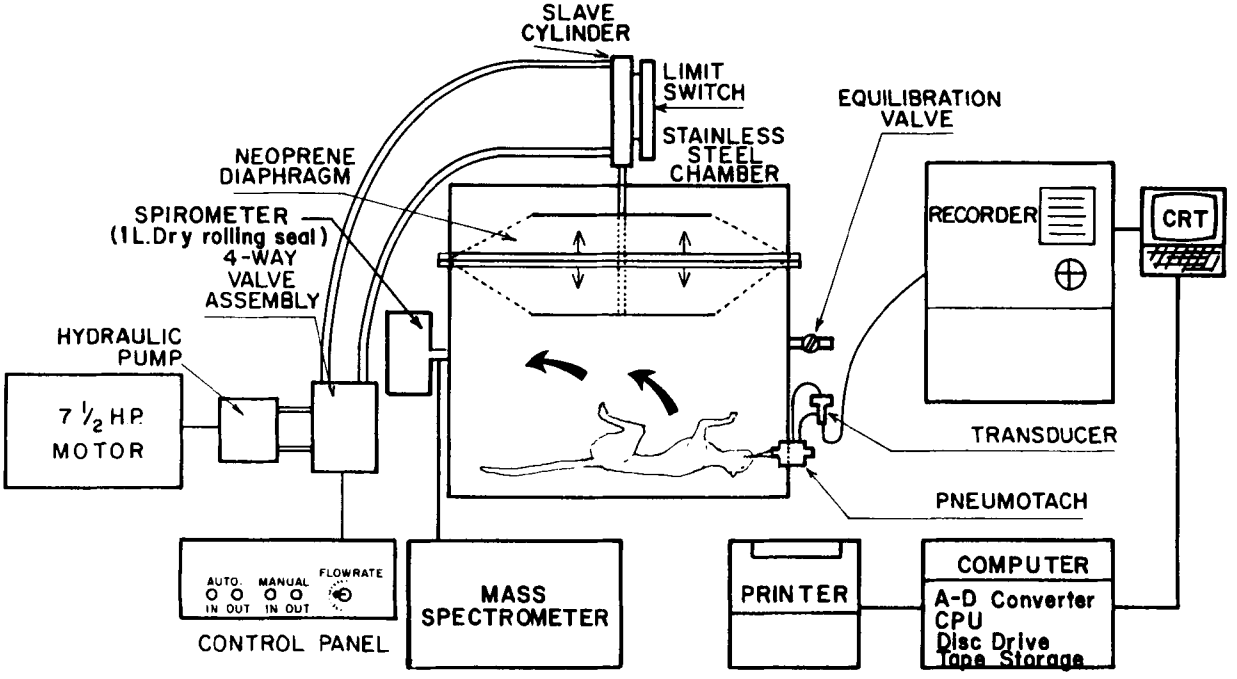

Figure 1. Diagramatic lay-out of pulmonary function laboratory.

particulate concentration of 6 mg m^{-3}. For the balance of the exposure period, it was adjusted to produce a concentration of 12 mg m^{-3}. The animals were exposed 8 hours per day, 7 days per week for the entire 2 years.

Pulmonary function testing was conducted following 20-24 h of no exposure. The cats were anesthetized with Ketaset Plus (Ketamine 100 mg ml^{-1} and acepromazine 7.5 mg ml^{-1}) at 42 mg kg^{-1} (Ketaset Plus Bristol Labs, Syracuse, NY) and a sterile, cuffed endotracheal tube (size 18-22 fr) was inserted into the trachea. To measure transpulmonary pressure, an esophageal balloon was inserted into the lower third of the esophagus and static pressure at the location of the mouth was sampled through a vertical tap on the endotracheal tube connector. The differential pressure was measured using a PM131TC Statham transducer (Statham Labs, Inc., Hato Rey, Puerto Rico). Airflow in and out of the intubated cat was measured with a pneumotachometer (Model No. 3075, Hans Rudolph Co., Kansas City, MO) and was transduced into an electrical signal using a PM-5 transducer (Statham Labs, Inc., Hato Rey, Puerto Rico). Airflow signals and transpulmonary pressure signals were recorded on a DR-12 photographic recorder (Electronics for Medicine, White Plains, NY). Volume was obtained by electrical integration of airflow with a variable time constant.

Pulmonary mechanical properties were measured during spontaneous breathing. Pulmonary flow resistance (RL ave. flow) was determined by analyzing the relationship between transpulmonary pressure and flow rate at equal lung volumes, and dynamic compliance (CL_{dyn}) was determined by relating the volume and pressure changes at points of zero flow as described by Frank *et al.*[9] The cats were supine for tests of mechanical properties.

Lung volumes, diffusion capacity, nitrogen washout and ventilatory performance were determined by using a whole-body plethysmograph-respirator, previously described by Moorman,[10] which provided the driving force required to produce breathing maneuvers for pulmonary function testing in anesthetized cats. All cats were placed in the prone position for this testing. A hydraulic system enabled the operator to control inspiration, expiration, breath holding and breathing rate. Lung volumes can be controlled by changing the pressure surrounding the cats and the rate of airflow in and out of the animal can be controlled by adjusting the rate of pressurization.

Total lung capacity (TLC) and the diffusion capacity for carbon monoxide (DL_{CO}) were determined by combining the methods of Brashear *et al.*[11] and Mitchell and Renzetti.[12] To determine TLC, the animal was first made apneic with a forced respiratory cycle, then the cat was slowly inspired maximally (with a test gas containing 10% helium and 0.3% $C^{18}O$). The inspiration was held for 10 s, then the animal was slowly expired maximally. During expiration, and end alveolar gas sample was collected and analyzed for helium (He) concentration. The He concentration was proportional to the volume into which it was diluted, and TLC was calculated as follows:

$$([He]_{initial}/[He]_{alveolar}) \times IC = TLC$$

The calculations for DL_{CO} were performed according to the method described for $C^{18}O$ by Wagner *et al.*[13]

Single-breath nitrogen tests were next performed according to methods described by Buist and Ross.[14] Following the induction of apnea, each cat was forced to slowly inspire maximally and then slowly forced to expire maximally. From residual volume, the animal breathes in 100% O_2, and the N_2 concentration of expired gas was measured continuously during expiration. From the resulting N_2 washout curve, closing volume (CV) and percent rise in N_2 at 25% of VC($\%N_{2\ 25\%VC}$) were measured.

Inspiratory capacity (IC) and forced vital capacity (FVC) were measured during forced vital capacity maneuvers accomplished by rapid depressurization (to −70 cm H_2O) and rapid repressurization (to +70 cm H_2O) of the plethysmograph. The resulting flow-volume curve demonstrate rapid development of peak flow, occurring prior to 50% of FVC. Flow maxima have been demonstrated by successive curves conducted at increasing plethysmograph pressures. The cat was maximally inspired with room air from end-tidal position, and the volume recorded as IC. FVC was then determined by measuring the volume expired when the plethysmograph was rapidly repressurized. After determining IC, FVC and TLC, expiratory reserve volume (ERV), residual volume (RV) and functional residual capacity (FRC) were calculated from standard formulae. Parameters of ventilatory performance were selected to detect obstructive changes in large central airways [peak expiratory flow rate (PEFR), forced expiratory volume in 0.5 s expressed as a percentage of FVC ($FEV_{0.5}$/FVC), forced expiratory flow at 50% of FVC ($FEF_{50\%\ FVC}$)] and small peripheral airways (forced expiratory flow at 25% of FVC ($FEF_{25\%\ FVC}$)].

All gas analyses were performed with a respiratory mass spectrometer (Model MGA 1100, Perkin-Elmer Corp., Norwalk, CT). Data sampling, storage and calculations were performed by a computer (CTP-5, Healthgarde, Inc., Salt Lake City, UT).

All data were tested statistically by non-parametric, Kruskal-Wallis one-way rank analysis of variance. The difference between control and exposed cats was tested following the first 61 weeks exposure period and again following 124 weeks of exposure.

RESULTS

Table 2 presents all parameters studied for control and exposed cats contrasting the values for the first 61 weeks with the balance (61-124 weeks). Following the first 61 weeks of exposure, no significant patterns of response were found in mechanical properties, diffusing capacity, uniformity of distribution or ventilatory performance. In contrast to these negative findings, a clearly defined response existed at the end of 124 weeks of exposure.

The reduction in inspiratory capacity, vital capacity and total lung capacity with normal values for dynamic ventilatory function (mechanics of breathing) indicates that a lesion is present which restricts breathing (volumes) but does not cause airway obstruction or loss of elasticity. This restrictive disease found in this study is compatible with a diagnosis of pulmonary fibrosis of the interstitial or intra-alveolar type. Concurrent status may include chronic inflammation, interstitial edema, or vascular engorgement. Additional support for the diagnosis of interstitial disease is the finding of impaired diffusing capacity. Distribution of this disease appears non-uniform as indicated by the significantly elevated nitrogen washout values for the exposed group.

Table 2. Mean (±SD) pulmonary function parameters comparing the control group with the diesel exposed group after 1 year and 2 years

	1 year		2 years	
Mechanical properties	Exposed	Control	Exposed	Control
CL_{dyn} (ml per cm H_2O)	23.5 ± 7.2	23.7 ± 0.3	27.5 ± 4.9	26.2 ± 7.1
$RL_{ave\ flow}$ (cm H_2O l^{-1} s^{-1})	10.7 ± 4.6	10.3 ± 4.4	8.6 ± 3.2	8.7 ± 2.3
Lung volumes (ml):				
TLC	415.0 ± 56.0	449 ± 74.5	428 ± 46.3[a]	484 ± 68.3
FVC	348 ± 43.5	369 ± 42.1	369 ± 42.3[a]	410 ± 57.6
FRC	158 ± 35.6	165 ± 42.2	145 ± 26.2[a]	163 ± 36.9
ERV	69 ± 24.6	67 ± 19.0	79 ± 24.0	83 ± 34.5
RV	86 ± 36.9	104 ± 37.7	67 ± 14.3	80 ± 28.2
RV/TLC (%)	20.3 ± 6.9	22.7 ± 5.9	15.6 ± 1.9	16.4 ± 4.5
IC	279 ± 44.8	301 ± 49.6	291 ± 44.1[a]	328 ± 58.6
Ventilatory performance:				
$FEV_{0.5}$ (%)	84.3 ± 8.4	81.6 ± 6.4	89.6 ± 6.1	86.9 ± 5.9
PEFR ($ml\ s^{-1}$)	1016 ± 185	1042 ± 174	887 ± 98[a]	952 ± 110.7
FEF_{50} ($ml\ s^{-1}$)	728 ± 196	761 ± 160	802 ± 125	864 ± 121
FEF_{25} ($ml\ s^{-1}$)	490 ± 186.8	481 ± 199.5	518 ± 154	574 ± 153
FEF_{10} ($ml\ s^{-1}$)	196 ± 107.4	222 ± 156.8	223 ± 109	234 ± 102
$FEF_{40\%}$ TLC ($ml\ s^{-1}$)	486 ± 252.6	447 ± 248.0	586 ± 173	625 ± 213
Diffusion (DLCO ($ml\ min^{-1}$ per mm Hg)	1.18 ± 0.43	1.22 ± 0.40	0.89 ± 0.27[a]	1.01 ± 0.14
Distribution and closing volume:				
$\%N_2$/25%/VC ($\%N_2$)	0.32 ± 0.20	0.29 ± 0.30	0.39 ± 0.27[a]	0.21 ± 0.18
CV (ml)	25.6 ± 13.4[a]	36.0 ± 16.1	27.0 ± 17.6	25.0 ± 19.3

[a] Statistically significant ($P < 0.05$).

DISUCSSION

Pathological description of pulmonary responses to diesel exhaust at similar concentrations has been previously characterized.[1,15] The observations include: (1) marked accumulation of black pigment laden macrophages in the interstitium localizing around blood vessels and respiratory bronchioles; (2) hyperplasia of the alveolar lining cells with focal thickening of the interstitium; (3) interstitial pneumonitis; (4) traces of, or no emphysema or peribronchiolitis.

The pathological findings from the cats in this study have been recently reported by Plopper *et al.*[16] They found the primary site of pulmonary damage to be the centriacinar region (transition zone between the distal conducting airways and the gas exchange area). The peribronchilar connective tissue space of the centriacinar region increased 2.7-fold in thickness. Considerable increases in connective tissue fibres, fibroblasts, interstitial macrophages and lymphocytes were located in this increased interstitium. Biochemical analysis demonstrated increased amounts of newly synthesized callogen. The airways proximal to terminal bronchioles did not demonstrate treatment effects. The pathological description is totally consistent with the pulmonary physiological interpretation, originally made in 1982,[7] of a restrictive pattern of reducing lung volume with normal airflow.

In conclusion, this report describes adverse pulmonary responses to high concentrations of diesel exhaust. While the levels studied are higher than actual environment or occupational exposures, the results may serve as a guide to the functional impairment resulting from considerably longer exposures likely to exist in human populations. While this report is the first to identify restrictive lung disease as a result of diesel exhaust exposure, it is not the only report describing the reduction of total lung capacity, inspiratory capacity and diffusing capacity which have been predictive of interstitial fibrotic pulmonary responses from diesel exhaust exposure.

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