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Comparative Pulmonary Toxicities and Carcinogenicities of Chronically Inhaled Diesel Exhaust and Carbon Black in F344 Rats

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Diesel exhaust (DE) is a known pulmonary carcinogen in rats, and the carcinogenic response is known to require the presence of soot. Many estimates of human lung cancer risk from inhaled DE have been developed from rat bioassay data or from the comparative mutagenic potencies of DE soot extract and known human chemical carcinogens. To explore the importance of the DE soot-associated organic compounds in the lung tumor response of rats, male and female F344 rats were exposed chronically to diluted whole DE or aerosolized carbon black (CB) 16 hr/day, 5 days/week at target particle concentrations of 2.5 mg/m^3 (LDE, LCB) or 6.5 mg/m^3 (HDE, HCB) or to filtered air. The CB served as a surrogate for the elemental carbon matrix of DE soot. Considering both the mass fraction of solvent-extractable matter and its mutagenicity in the Ames *Salmonella* assay, the mutagenicity in revertants per unit particle mass of the CB was three orders of magnitude less than that of the DE soot. Both DE soot and CB particles accumulated progressively in the lungs of exposed rats, but the rate of accumulation was higher for DE soot. In general, DE and CB caused similar, dose-related, nonneoplastic lesions. CB and DE caused significant, exposure concentration-related increases, of similar magnitudes, in the incidences and prevalences of the same types of malignant and benign lung neoplasms in female rats. The incidences of neoplasms were much lower in males than females, and the incidences were slightly higher among DE- than CB-exposed males. Survival was shortened in the CB-exposed males, and the shortened survival may have suppressed the expression of carcinogenicity as measured by crude incidence. Logistic regression modeling did not demonstrate significant differences between the carcinogenic potencies of CB and DE in either gender. The results suggest that the organic fraction of DE may not play an important role in the carcinogenicity of DE in rats.

Diesel exhaust (DE) is a complex mixture of gases, vapors, and soot particles. The soot consists of respirable, chain aggregate, elemental carbon particles having a high specific surface area to which a mixture of organic compounds is adsorbed. The organic fraction, which contains more than 450 compounds, including known mutagens and carcinogens (Opresko *et al.*, 1984), typically constitutes from 5 to 30% of the total soot mass.

DE is a pulmonary carcinogen in rats (reviewed in Mauderly, 1992). The lung tumor incidence is increased in a dose-related manner in rats exposed repeatedly for 24 months or longer to DE at weekly soot exposure rates (concentration × time) above approximately 120 mg · m^{-3} · hr (e.g., 4 mg/m^3, 6 hr/day, 5 days/week). Filtered DE is not a pulmonary carcinogen in rats, thus demonstrating that the carcinogenic response requires the presence of soot (Heinrich *et al.*, 1986; Brightwell *et al.*, 1989).

Several studies have suggested that the lung tumor response of rats to chronic DE inhalation might be attributable to the initiating action of genotoxic metabolites of the soot-associated organic compounds. The solvent-extracted organic fraction of DE is mutagenic in the Ames *Salmonella* assay (Claxton, 1983). DE soot-associated organic compounds, including the carcinogens benzo(*a*)pyrene and nitropyrene, can be eluted from soot after deposition in the rat lung (Sun *et al.*, 1988). Wong *et al.* (1986) demonstrated that the group mean total lung DNA adduct level from a group of DE-exposed rats having an increased tumor incidence was increased above that of controls, suggesting that soot-borne organic compounds are released in the lung and are genotoxic.

Other evidence, however, suggests that the carcinogenicity of DE in rats might reflect a nonspecific response of the species to the accumulation of the relatively insoluble particles in the lung and might have little to do with the organic fraction. Intratracheally instilled activated carbon particles (Kawabata *et al.*, 1986) or inhaled particles without a bioavailable organic fraction, such as titanium dioxide (Heinrich, 1994), nonasbestiform talc (NTP, 1994), and oil shale (Holland *et al.*, 1986), can cause lung cancer in rats. Vostal (1986) noted that the lung cancer response to DE occurs only in rats exposed at sufficient rates to cause a progressive lung accumulation of soot and an accompanying complex of inflammation, epithelial hyperplasia, meta-

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plasia, and fibrosis and that the lower exposures of humans were unlikely to produce these effects. These observations led to uncertainty about the mechanism by which DE caused lung tumors in rats and speculation that the rat bioassay results might not be predictive for human cancer risk (Mauderly *et al.*, 1990).

Epidemiological studies have attempted to determine human cancer risk from DE exposures (reviewed in Mauderly, 1992, 1995). Case–control (Garshick *et al.*, 1987) and retrospective cohort (Garshick *et al.*, 1988) studies indicated relative risks on the order of 1.4 for lung cancer among railroad workers with long-term exposures to DE. After reviewing the available *in vitro*, bioassay, and epidemiological data, the U.S. National Institute for Occupational Safety and Health classified DE as a "potential occupational carcinogen" (NIOSH, 1988). After reviewing essentially the same data, the International Agency for Research on Cancer and the U.S. Environmental Protection Agency each classified DE as a "probable human carcinogen" (IARC, 1989; EPA, 1990).

It became critical to determine whether the soot-associated organic compounds were important in the lung cancer response of rats. Most estimates of unit lung cancer risk for humans have been developed from extrapolation of the rat bioassay data or from the comparative mutagenic potencies of DE soot extract and known human chemical carcinogens (Mauderly, 1992). The former requires confidence that the mechanism by which DE induces lung tumors in rats would also occur in humans, and the latter assumes that the genotoxicity of the soot-associated organic fraction is responsible for carcinogenesis.

This study explored the importance of the DE soot-associated organic compounds in the lung tumor response of rats by comparing responses in rats exposed chronically to whole DE and aerosolized carbon black (CB) at identical particle concentrations. The CB served as an approximate surrogate for the elemental carbon matrix of organic-free DE soot. Because comparative carcinogenic potency was the primary concern, rather than exposure–response relationships, exposure levels were selected for which it was certain that DE would induce significant increases in pulmonary cancer. Because the carcinogenicity of inhaled CB was unknown, two exposure levels were used to increase the probability that the carcinogenicities of DE and CB could be adequately compared. Lung burdens of soot and CB particles were measured at selected times during the study to provide an additional comparative dose term for relative carcinogenicity. The carcinogenicity was compared on the basis of the occurrence of lung neoplasms, the prevalence of neoplasms with time, and types of neoplasms induced.

MATERIALS AND METHODS

Animals, maintenance, and exposure. Female and male, 5- to 7-week-old, F344/N rats from the barrier-maintained, specific-pathogen-free, ITRI production colony were acclimated to inhalation exposure chambers (2 m^3 volume, H2000, Lab Products, Inc., Maywood, NJ) ventilated with filtered air for 2 weeks. Midway through the acclimation period, one rat of each gender was selected randomly from each acclimation chamber and necropsied; serum was collected for serological surveillance for pathogens (Standard Level II Antibody Profile, Microbiological Associates, Rockville, MD). The surveillance included pneumonia virus of mice, reovirus Type 3, Sendai virus, lymphocytic choriomeningitis virus, Kilham rat virus, Toolan H-1, rat corona virus/SDA, *Mycoplasma pulmonis*, and CAR Bacillus. The rats were assigned to five treatment groups by randomizing each gender by body weight measured 9 days before the start of exposures. The rats were 7 to 9 weeks old when the exposures began. Approximately 100 rats of each gender per treatment group were observed for life span to evaluate body weight, survival, and carcinogenicity (Table 1). The exposures were terminated at 24 months, and the remaining rats were transferred to an animal housing room where they were maintained for an additional 6 weeks. At that time, mortality had reached approximately 90%, and the remaining rats were euthanized.

The rats were housed in individual wire cages within the exposure chambers for the 24-month exposures. Although aerosols were distributed uniformly throughout these chambers (Griffis *et al*, 1981), the cages were rotated once weekly in a clockwise direction to ensure uniform exposure of the rats. There were two chambers per treatment group. Males and females were housed in the same chamber. Airflow in the exposure chambers was 425 ± 57 liters per minute, providing about 15 air changes per hour. The relative humidity was 40 to 70%, and the temperature was 23 to 25°C. The exposure chambers were washed weekly. Bacteriostatic liners (Shepherd Specialty Papers, Inc., Kalamazoo, MI) in excreta trays below each cage unit were changed twice daily, and the trays were washed daily. After 24 months, surviving rats were transferred from the exposure chambers to an animal housing room where they were maintained in plastic cages with hardwood chip bedding (P. J. Forrest Products Corp., Montville, NJ) and filter tops. The cages were washed weekly. Relative humidity was 14 to 36%, and room temperature was 21 to 25°C. Throughout the study, standard fluorescent lighting was on a 12-hr cycle (on 0600–1800). A pelleted ration (Certified Wayne Lab Blox, Allied Mills, Chicago, IL) and tap water delivered through automatic water valves were available at all times. Because of the length of the daily exposures (16 hr), the food trays were not removed during the exposures. The rats were observed for morbidity and mortality twice daily and weighed monthly until 22 months and biweekly thereafter.

TABLE 1

Treatment Groups of F344 Rats Used to Compare Pulmonary Toxicities and Carcinogenicities of Diesel Exhaust and Carbon Black

Exposure group	Target particle concentration (mg/m^3)	Number of rats entered into exposure chambers[a]		
		Female	Male	Total
Low carbon black (LCB)	2.5	116	115	231
High carbon black (HCB)	6.5	114	115	229
Low diesel exhaust (LDE)	2.5	114	114	228
High diesel exhaust (HDE)	6.5	115	115	230
Sham-exposed control (C)	0	114	118	232
Total		573	577	1150

[a] Six (three female and three male) rats per exposure group were sacrificed after 3, 6, 12, 18, and 23 months of exposure. Approximately 100 rats of each gender per exposure group were maintained for life span.

The rats were exposed 16 hr/day, 5 days/week (except holidays) to diluted whole DE or CB aerosolized in air at target particle concentrations of 2.5 mg/m^3 (LDE, LCB) or 6.5 mg/m^3 (HDE, HCB) or to filtered (high efficiency particulate (HEPA) filters) ambient air as sham-exposed controls (C). DE was generated using two 1988 Model LH6 General Motors 6.2-liter V-8 engines burning D-2 control fuel that met EPA certification standards. The engines were alternated every 2 to 3 months. One engine was used for a total of 3824 hr and the other for 5284 hr. The engines were fitted with four-speed automatic transmissions, mounted on test stands, and operated continuously by computer on the Federal Test Procedure urban certification cycle during exposures (U.S. Code of Federal Regulations, 1981). The system was calibrated to simulate operation of a Chevrolet C1500 pickup having a gross weight of 2773 kg. Exhaust passed through a standard exhaust system, including muffler and tailpipe, and was diluted serially with filtered air to the desired soot concentration (Mokler *et al.*, 1984). The CB (Elftex-12 furnace black, Cabot, Boston, MA) was aerosolized using air jet dust generators (Jet-O-Mizer Model 0101, Fluid Energy, Hatfield, PA) and diluted with filtered air to the desired particle concentrations.

The particle concentration in each DE and CB chamber was measured gravimetrically by daily filter samples. The particle size distribution was measured at the beginning, midpoint, and end of exposures using a serial sampling train consisting of a cascade impactor in series with a parallel-flow diffusion battery (Cheng *et al.*, 1984). Samples of DE soot and CB were extracted twice using dichloromethane and sonication (Bechtold *et al.*, 1984). A total of 128 filter samples collected from the HDE chambers at different times during the study were extracted. Two or more filters were extracted together, and the extracts from larger numbers of filters were combined for final drying and weighing. Because of the low mass fraction of organic material on CB, bulk samples, rather than filter samples, were extracted at four different times during the study. Extracts obtained near the end of the study were used to assay mutagenicity. The bacterial mutagenicities of the solvent extracts were measured by an independent laboratory (Microbiological Associates, Rockville, MD) using a modified Ames *Salmonella* assay (Ames *et al*., 1975), three dilutions of each extract, and tester strains TA98 and TA100 with and without metabolic activation with rat liver S-9 microsomal fraction. Each assay was done in triplicate. Gas samples collected in Teflon bags were taken weekly, alternately from one of the two chambers at each exposure level for DE and CB for analysis of gases and vapors. Background concentrations of particles, vapors, and gases were measured in the C chambers.

Serial sacrifices, lung weights, and lung burdens. Rats were included in each exposure group for interim sacrifices. Three females and three males were selected randomly, anesthetized with halothane, and sacrificed by exsanguination after 3, 6, 12, 18, or 23 months of exposure. At sacrifice, blood was collected from one male and one female rat from each chamber for serological surveillance. The lungs were weighed in aggregate and as individual lobes. The left lung was used for measurement of particle burden, the right diaphragmatic lobe was fixed for histopathology, and the remaining lobes were retained for other uses.

To estimate the amount of DE soot or CB in the left lungs, the lungs were homogenized in saline, and the extinction of light at 620 nm wavelength by homogenates was compared to standard curves constructed from lung tissue samples to which known amounts of particles were added (Henderson *et al.*, 1987). The lung burdens should be considered estimates because the light extinction assay is dependent not only on the concentration of particles in the homogenates but also on the degree of dispersion of the particles. The accuracy of the values is dependent on the degree to which dispersion of the particles in the homogenized spiked samples resemble that in the homogenized exposed lungs. Left lung particle burdens were extrapolated to total lung burdens by lung weight.

Necropsy and histopathology. All rats received a complete necropsy. The right diaphragmatic lung lobe of sacrificed rats was fixed for 4 to 6 hr by bronchial perfusion at a constant hydrostatic pressure of 20 cm of 10% neutral buffered formalin or, at the 23-month sacrifice, with 4% buffered paraformaldehyde. Lungs of rats that died or were euthanized (nonsacrificed) were fixed by intratracheal instillation of fixative. After perfusion or instillation of fixative, the airway was ligated, and the lung was submerged in a large volume of fixative for at least 24 hr. For nonsacrificed rats, four sagittal sections of lung, one each from the right apical, right cardiac, and right diaphragmatic lobes, and one from the left lung were cut so as to include the main axial airway. These sections were paraffin embedded, cut at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy. For sacrificed rats, five or six transverse sections of the right diaphragmatic lobe were processed in the same way and evaluated. Additional sections were taken from all rats as needed to evaluate lung or thoracic lesions noted at necropsy.

Histopathologic findings were entered into a computer data base (PathTox, Xybion Medical Systems, Cedar Knolls, NJ) using standardized terminology. Neoplastic and nonneoplastic lesions were scored as present or absent. If present, then the severity of each nonneoplastic lesion, except squamous cysts, was graded on a scale of one to four, indicating the fraction of the lung or structure involved and the intensity of the reaction.

Statistical evaluations. The criterion for statistical significance was set at $p < 0.05$ for all analyses. Survival curves were generated using Kaplan–Meier curves (Kalbfleisch and Prentice, 1980) and differences among survival patterns were tested using the log-rank test (Harrington and Flemming, 1982). The significances of differences in lung weight were analyzed by multiple pairwise comparisons with a two-sided t test. The t values were derived using separate (rather than pooled) variances, and the Bonferroni correction was used to adjust t values for multiple tests.

The influences of exposure material and exposure level on the development of nonneoplastic lung lesions were examined using a prevalence model. The data from the rats that were sacrificed were combined with those from rats that died or were euthanized. The severity scores for the nonneoplastic lesions except squamous cysts ranged from 0, which meant that the lesion was not present, to 4, which meant that approximately 50% or more of the lung was involved. Scores like these can be analyzed using polychotomous logistic regression, a technique for analyzing multinomial ordinal data with more than two outcomes. Ordinal data, such as these scores, are ordered categories, and the spacing between the categories does not have to be equal. In this technique the cumulative distribution probabilities of the scores are modeled, i.e., the probability of a score and all lower scores. A linear model of the logit of the cumulative probabilities is estimated. The simplest model of parallel regressions for scores was used (McCullagh and Nelder, 1983).

The nonneoplastic lesions in this study were rare in the controls, and the prevalences, even at the low exposure levels, were always much higher in the exposed animals for both DE and CB. Because the nonneoplastic lesions were rare in the controls, the polychotomous logistic regression model was used only to estimate the effects of exposure concentration and type of exposure (DE versus CB). A separate model was estimated for each gender with covariates of time on study, type of exposure, and exposure level based upon exposure concentration.

A logistic regression model like the model used for the lung neoplasms (described below) was used for the squamous cysts, because the cysts were tabulated as present or absent in the lung of each animal.

Prevalence of neoplasia, the probability that a rat living at a given time had a benign or malignant lung neoplasm, was calculated using data from rats sacrificed, euthanized, or dying within selected time intervals during the study. The prevalence of lung neoplasms was considered a better measure of carcinogenicity than the crude incidence because most of the neoplasms were incidental findings and because survival was altered by the exposure concentration and type of exposure. Prevalences of neoplasms were also modeled using a logistic regression approach to examine significances of group differences in lung neoplasm response (McKnight and Crowley, 1984). Because the neoplasms did not appear to alter the time of death, the times of sacrifice, death, or euthanasia were regarded as provid-

ing random samples of times at which rats were examined for neoplasms (Dinse and Lagakos, 1983). The statistical design of this study has the factors of gender crossed with the two materials, DE and CB, and two exposure concentrations. The data were analyzed separately for each gender. The model for each gender included a term for time on study to account for the increasing incidence of lung tumors with age and terms for exposures to DE and CB to describe the dose response to each exposure material. The exposure terms were the product of the estimated exposure concentrations and the number of days on study. The model for each gender was

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right) = \alpha + \beta t + \gamma C_{de} + \delta C_{cb},$$

where p was the prevalence, t was the time on study in days, C_{de} was the product of diesel exhaust exposure concentration in units of mg/m^3 and time in days (this product was 0 for controls and rats exposed to carbon black), C_{cb} was the product of carbon black exposure concentration in units of mg/m^3 and time in days (0 for controls and rats exposed to diesel exhaust), and α, β, γ, and δ were the coefficients of the model that were estimated by logistic regression.

RESULTS

Exposures. The exposure atmospheres are summarized in Table 2. Comparisons, using Student's t test, of exposure material concentrations between the four pairs of exposure chambers yielded p values ranging from 0.23 to 0.88. Because the atmospheres did not differ between the two chambers for each exposure group, these data are combined in Table 2. Particle concentrations were very close to the target values and varied little during the study.

Particle size was similarly bimodal for DE and CB. The mass median aerodynamic diameter (and geometric standard deviation, GSD) of the large-size mode was 2.00 μm (2.09) and 1.95 μm (1.84) for DE and CB, respectively. The mass median diffusion diameter of the small-size mode was 0.10 μm for both DE and CB, with GSDs of 2.15 and 2.16 for DE and CB, respectively. The portions of particles in the larger and smaller modes differed between the two exposure materials, however, with approximately 23% by mass in the larger mode for DE and 67% in the larger mode for CB.

For the engines used for 58 and 42% of the exposure days, the mean mass fractions of extractable organic material on DE soot were 7.1% (min–max values from combined samples = 6.2–8.6%) and 9.8% (min–max values from combined samples = 9.1–10.5%), respectively. The weighted mean extractable fraction for exposures to DE soot was 8.2%. The mean value from the four extractions of CB was 0.12%, with individual values ranging from 0.04 to 0.29%. The DE soot, therefore, contained approximately 66 times more extractable organic matter than CB.

As observed previously (Clark and Vigil, 1980; Bechtold *et al.*, 1984), the mutagenicity of DE soot extracts was higher in tester strain TA100 than in strain TA98 and slightly higher without metabolic activation by rat liver S-9 microsomal fraction than with activation. Very little mutagenic activity was associated with CB extract. The specific mutagenic activities of the extracts in strain TA98, calculated from the mean responses (three dilutions of each extract, each repeated in triplicate) after subtracting the background response to the vehicle control, were 0.25 revertants per microgram for DE and 0.003 for CB, an 83-fold difference. The specific activities in strain TA100 were 0.68 revertants per microgram for DE and 0.017 for CB, a 40-fold difference. Combining the differences in extractable fraction and specific mutagenicity yielded a mutagenicity in revertants per unit of particle mass that was approximately 5500 times higher for DE than for CB in strain TA98 and 2600 times higher in TA100.

Clinical signs. No clinical signs related to exposure other than darkened fur coats and decreased body weight gains were observed. The antibody titers for viruses, mycoplasma, and bacteria were negative throughout the study.

Survival and body weight. The survival of the rats differed significantly among the exposure groups. Estimates of

TABLE 2

Concentrations[a] of Key Constituents of Exposure Atmospheres Collected at Chamber Midpoint during 24 Months of Exposure

		Carbon black		Diesel exhaust		
		LCB	HCB	LDE	HDE	C
Constituent	Units	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
Total particles	mg/m^3	2.46 ± 0.03	6.55 ± 0.06	2.44 ± 0.02	6.33 ± 0.04	0.05 ± 0.02
Carbon monoxide	ppm	0.70 ± 0.06	0.69 ± 0.06	10.30 ± 0.23	26.85 ± 0.52	0.78 ± 0.06
Carbon dioxide	ppm	2010 ± 64	1820 ± 65	4470 ± 77	7390 ± 87	2210 ± 58
Hydrocarbon vapors	ppm	4.37 ± 0.09	4.21 ± 0.08	6.47 ± 0.15	8.13 ± 0.20	4.53 ± 0.08
Total oxides of nitrogen	ppm	0.030 ± 0.004	0.033 ± 0.004	8.79 ± 0.32	23.45 ± 0.69	0.033 ± 0.003
Nitrogen dioxide	ppm	0.027 ± 0.004	0.029 ± 0.004	0.73 ± 0.05	3.78 ± 0.18	0.023 ± 0.002

[a] Values are means ± SE of weekly mean values for particles and weekly values for vapors and gases. Values for diesel exhaust and carbon black groups include background concentrations of particles, gases, and vapors listed for sham exposures.

the survival curves of each gender and treatment grc after 400 days of exposure are shown in Fig. 1. Males h consistently shorter survival times than females, but relationships between survival and exposure differed tween females and males. As can be seen in Fig. 1, the s vival patterns for female groups cross each other in a co plex manner, whereas the fractions of surviving male L and HCB rats were consistently lower than those of the males after approximately 500 days, and the survival of HDE males was reduced after approximately 600 days. T differences in survival were particularly evident in ma late in the exposures. At 23 months, 13.8% of the C mal 4.3 and 0.7% of the LCB and HCB males, and 14.4 a 5.8% of the LDE and HDE males, respectively, were ali At 23 months, 35.6% of the C females, 40.4 and 25.9% the LCB and HCB females, and 30.9 and 26.7% of the LI and HDE females, respectively, were alive.

The mean body weights of all living rats at each weighi time are presented in Fig. 2. Weights were persistently fected by all treatments except the LDE exposures.

Lung burdens of particles and lung weights. The lu burdens of particles in female and male rats sacrificed af 3, 6, 12, 18, and 23 months of exposure are shown in Ta

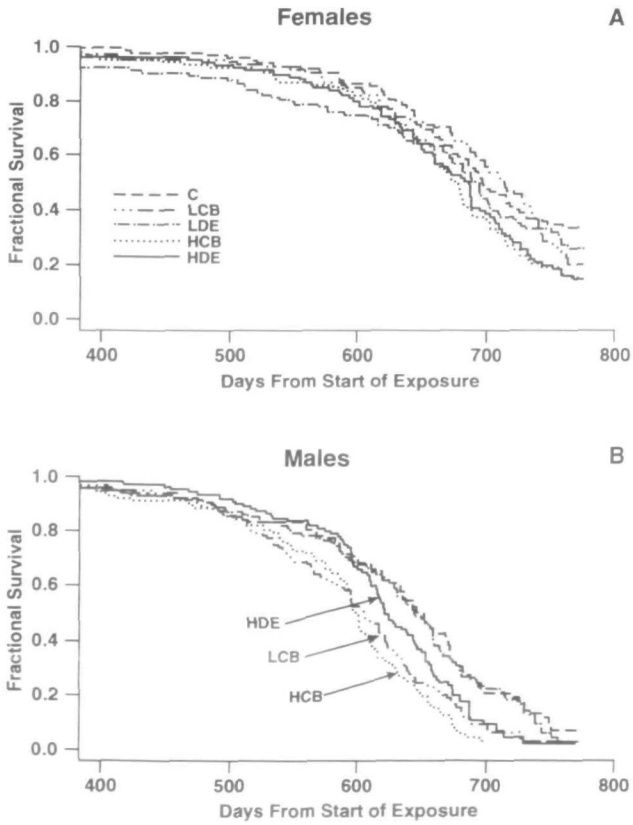

FIG. 1. The survival of female (A) and male (B) rats after 400 days of exposure is illustrated by curves generated using the Kaplan–Meier method (Kalbfleisch and Prentice, 1980).

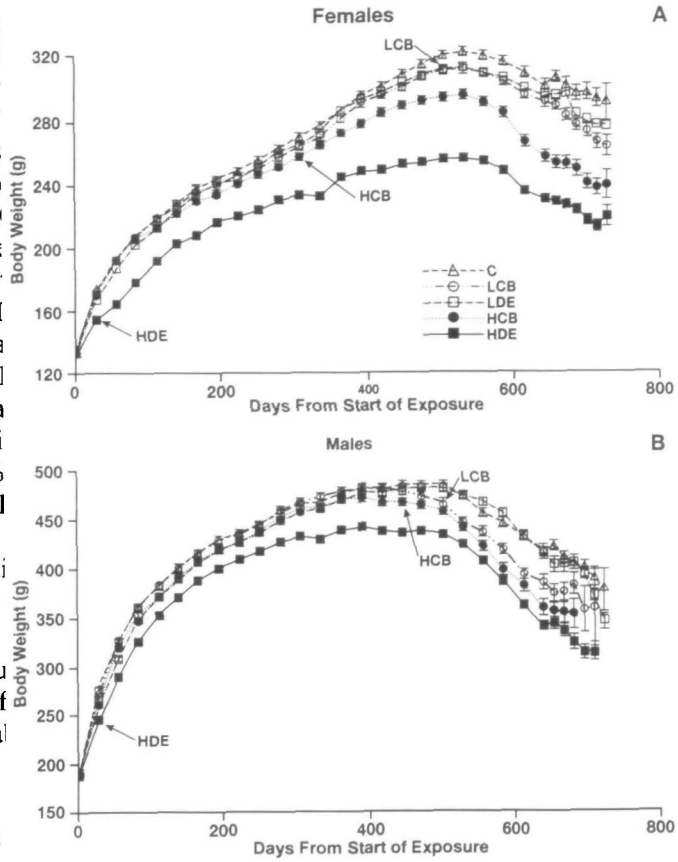

FIG. 2. The body weights of female (A) and male (B) rats in the five exposure groups throughout the study are illustrated. Values represent means ± SE for gender-specific group sizes that varied from 139 at the first weighing to 2 at the last. Arrows indicate the time at which the respective group mean value became significantly different from the control mean at $p < 0.05$.

3. The progressive accumulation of DE soot and CB particles tended to accelerate after 12 months of exposure, and DE soot accumulated more rapidly than CB. In both female and male rats at 18 and 23 months of exposure, the lung burdens of particles were similar in the LDE and HCB groups. At 23 months, mean lung burdens of the LCB and HCB female rats had reached 17.3 and 36.9 mg, respectively, and those of the LDE and HDE females had reached 36.7 and 80.7 mg, respectively. At 23 months, mean lung burdens of the LCB and HCB male rats had reached 24.7 and 40.1 mg, respectively, and those of the LDE and HDE males had reached 45.1 and 90.1 mg, respectively.

The lung weights of all exposure groups of both genders (Table 4) increased in a nearly linear manner throughout the exposure. Significant increases in lung weight above the C mean value occurred at 3 and 6 months for the HCB and HDE females, respectively, and at 6 and 12 months for the LCB and LDE females, respectively. Significant increases in lung weight above the C mean value occurred at 12 and 6 months for the HCB and HDE males, respectively, and at

TABLE 3
Lung Burdens of Particles in Female and Male Rats Sacrificed after 3, 6, 12, 18, and 23 Months of Exposure

	3 Months		6 Months		12 Months		18 Months		23 Months	
Group	F	M	F	M	F	M	F	M	F	M
LCB	1.7 ± 0.2[a]	1.7 ± 0.2	3.3 ± 0.5	5.6 ± 1.6	6.2 ± 0.5	7.9 ± 1.1	12.1 ± 1.2	16.0 ± 1.1	17.3 ± 3.6	24.7 ± 2.4
HCB	4.9 ± 0.3	5.9 ± 0.2	11.0 ± 0.4	13.7 ± 1.7	12.2 ± 2.5	15.1 ± 0.5	22.7 ± 1.6	29.9 ± 1.3	36.9 ± 6.8	40.1 ± 1.8
LDE	2.8 ± 0.8	2.5 ± 0.7	4.9 ± 1.8	6.5 ± 0.4	9.8 ± 3.2	12.4 ± 1.6	21.7 ± 1.6	29.3 ± 2.5	36.3 ± 7.5	45.1 ± 7.4
HDE	4.7 ± 1.5	7.0 ± 0.2	18.9 ± 1.8	20.8 ± 1.5	20.8 ± 8.7	28.3 ± 4.0	41.2 ± 5.0	58.0 ± 6.9	80.7 ± 4.5	90.1 ± 4.1

[a] Values are milligrams of carbon black or diesel soot per whole lung (mean ± SD, $n = 3$).

12 and 18 months for the LCB and LDE males, respectively. Because of variability, the significant difference was not always sustained, particularly in the low exposure groups.

Nonneoplastic morphologic responses. Exposure-related lesions consisted of alveolar macrophage hyperplasia, alveolar epithelial hyperplasia, chronic-active inflammation, septal fibrosis, alveolar proteinosis, bronchiolar-alveolar metaplasia, focal fibrosis with alveolar epithelial hyperplasia, squamous metaplasia, and squamous cysts. These lesions are described below in approximate order of occurrence. Tables 5 and 6 show the percentages of female and male rats with each nonneoplastic lesion and the range of severity scores at two time intervals.

Alveolar macrophage hyperplasia. At 3 months, increased numbers of enlarged macrophages containing particles were found scattered throughout the lungs of all exposed rats. In the LCB and LDE groups, there was minimal tendency toward localization of the macrophages in the centriacinar region. Localization was more pronounced in the HDE and, particularly, the HCB groups. At later sacrifices, the aggregation of macrophages in the centriacinar region was pronounced in all exposed rats. The main qualitative difference between the DE- and CB-exposed rats was that the zone of macrophage hyperplasia and aggregation was more tightly localized to the centriacinar region in the CB-exposed rats, while in DE-exposed rats, the zone of involvement was larger. At 3 months, only a few particle-containing macrophages were located in the interstitium of the centriacinus. The number and size of interstitial macrophages and macrophage aggregates had markedly increased by 12 months of exposure and continued to increase with exposure duration. However, at all sacrifices, the macrophages were predominantly located within the lumens of alveolar ducts and alveoli.

Alveolar epithelial hyperplasia. Alveolar epithelial hyperplasia consisted of an increased number of hypertrophic, cuboidal, alveolar epithelial cells lining alveolar septa. This lesion was most often colocalized with the alveolar macrophage hyperplasia. Alveolar epithelial hyperplasia was present in the lungs of most exposed rats by 3 months and in all exposed rats by 6 months. The severity of the lesion increased with exposure concentration and duration. As with the macrophage hyperplasia, the main difference between the DE- and CB-exposed rats was that the zone of involvement was less restricted to the centriacinar region and involved more of the lung in the DE-exposed rats, especially the HDE rats.

At 18 months of exposure and later, particularly in the HDE and HCB groups, the epithelial hyperplasia in some foci differed in character from that seen earlier. The amount of alveolar epithelial hyperplasia was increased relative to the amount of macrophage hyperplasia and septal fibrosis. Also, in some foci, there were micropapillary pro-

TABLE 4
Lung Weights of Female and Male Rats Sacrificed after 3, 6, 12, 18, and 23 Months of Exposure

	3 Months		6 Months		12 Months		18 Months		23 Months	
Group	F	M	F	M	F	M	F	M	F	M
C	0.94 ± 0.08[a]	1.32 ± 0.02	1.03 ± 0.04	1.40 ± 0.07	1.09 ± 0.05	1.58 ± 0.13	1.45 ± 0.44	1.77 ± 0.03	1.25 ± 0.09	1.99 ± 0.19
LCB	1.06 ± 0.01	1.35 ± 0.14	1.17 ± 0.06[b]	1.71 ± 0.15	1.59 ± 0.06[b]	2.17 ± 0.22[b]	2.01 ± 0.08	2.69 ± 0.69	2.42 ± 0.31[b]	3.12 ± 0.58
HCB	1.21 ± 0.03[b]	1.56 ± 0.16	1.68 ± 0.11[b]	2.12 ± 0.36	2.56 ± 0.07[b]	3.31 ± 0.34[b]	3.71 ± 0.44[b]	3.50 ± 0.11[b]	4.95 ± 0.13[b]	4.48 ± 1.48
LDE	1.02 ± 0.02	1.31 ± 0.14	1.15 ± 0.08	1.49 ± 0.13	1.67 ± 0.20[b]	2.03 ± 0.39	2.24 ± 0.12	2.52 ± 0.12[b]	2.91 ± 0.07[b]	3.26 ± 0.38[b]
HDE	1.03 ± 0.08	1.54 ± 0.12	1.74 ± 0.19[b]	2.10 ± 0.19[b]	3.18 ± 0.08[b]	3.95 ± 0.12[b]	4.59 ± 0.14[b]	5.17 ± 0.37[b]	5.86 ± 0.56[b]	5.98 ± 0.37[b]

[a] Values are grams (mean ± SD, $n = 3$).
[b] Significantly different from control rats of the same sex sacrificed at the same time.

TABLE 5
Percentages of Female Rats with Nonneoplastic Lung Lesions

Lesion	Control		LCB		HCB		LDE		HDE	
	<18 Months[a]	>18 Months[b]	<18 Months	>18 Months	<18 Months	>18 Months	<18 Months	>18 Months	<18 Months	>18 Months
Alveolar macrophage hyperplasia	0 (0–0)	4 (0–1)	100 (1–3)	100 (2–4)	96 (0–4)	100 (3–4)	100 (1–4)	100 (2–4)	96 (0–4)	100 (3–4)
Alveolar epithelial hyperplasia	0 (0–0)	9 (0–2)	90 (0–3)	100 (1–4)	93 (0–4)	100 (2–4)	88 (0–4)	100 (1–4)	96 (0–4)	100 (2–4)
Chronic-active inflammation	0 (0–0)	5 (0–1)	24 (0–2)	34 (0–2)	37 (0–3)	63 (0–2)	30 (0–2)	49 (0–2)	42 (0–2)	72 (0–3)
Septal fibrosis	0 (0–0)	2 (0–1)	52 (0–3)	96 (0–3)	78 (0–3)	100 (1–4)	61 (0–4)	93 (0–4)	73 (0–4)	98 (0–4)
Alveolar proteinosis	0 (0–0)	0 (0–0)	14 (0–1)	27 (0–2)	56 (0–3)	99 (0–4)	27 (0–3)	83 (0–4)	69 (0–4)	100 (1–4)
Bronchiolar-alveolar metaplasia	0 (0–0)	1 (0–2)	29 (0–2)	66 (0–3)	52 (0–3)	97 (0–4)	45 (0–3)	89 (0–4)	65 (0–4)	99 (0–4)
Focal fibrosis with epithelial hyperplasia	0 (0–0)	0 (0–0)	0 (0–0)	17 (0–4)	7 (0–2)	31 (0–3)	6 (0–2)	16 (0–3)	0 (0–0)	49 (0–3)
Squamous metaplasia	0 (0–0)	0 (0–0)	0 (0–0)	6 (0–2)	0 (0–0)	24 (0–3)	0 (0–0)	6 (0–2)	0 (0–0)	20 (0–3)
Number of rats	23	91	21	95	27	87	33	81	26	89

[a] Percentage (and range of severity scores) of female rats dying, euthanized, or sacrificed before 18 months of exposure that had each nonneoplastic lung lesion.

[b] Percentage (and range of severity scores) of female rats dying, euthanized, or sacrificed after 18 months of exposure that had each nonneoplastic lung lesion.

jections of hyperplastic epithelium, or there was a multilayering of the hyperplastic epithelial cells.

Chronic-active inflammation. The term chronic-active inflammation was used to denote the presence of focal aggregates of lumenal or interstitial neutrophils, in addition to alveolar macrophages, accompanied by evidence of cell injury in the form of degenerate inflammatory cells or cell debris. Although a few neutrophils were evident among the particle-laden macrophages at earlier times, chronic-active inflammation, as used here, was not observed in most exposed rats until approximately 12 months. By that time, cholesterol clefts, by-products of necrosis, were prominent in the lesions scored as chronic-active inflammation. The severity of chronic-active inflammation increased with exposure concentration and duration.

Septal fibrosis. Interstitial fibrosis observed within alveolar septa was diagnosed as septal fibrosis. Septal fibrosis mostly occurred in foci where macrophages were aggregated in the lumen or interstitium and where there was concomitant alveolar epithelial hyperplasia. Although septal fibrosis was observed at earlier times, it did not become a prominent lesion until after 12 months of exposure. The

TABLE 6
Percentages of Male Rats with Nonneoplastic Lung Lesions

Lesion	Control		LCB		HCB		LDE		HDE	
	<18 Months[a]	>18 Months[b]	<18 Months	>18 Months	<18 Months	>18 Months	<18 Months	>18 Months	<18 Months	>18 Months
Alveolar macrophage hyperplasia	3 (0–2)	0 (0–0)	100 (1–3)	100 (1–3)	10 (2–4)	100 (2–4)	100 (1–4)	100 (2–4)	100 (2–4)	100 (3–4)
Alveolar epithelial hyperplasia	0 (0–0)	2 (0–2)	98 (0–4)	100 (1–3)	100 (1–4)	100 (1–4)	94 (0–4)	100 (2–4)	97 (0–4)	100 (1–4)
Chronic-active inflammation	0 (0–0)	1 (0–3)	9 (0–2)	14 (0–2)	20 (0–2)	34 (0–3)	12 (0–1)	23 (0–2)	50 (0–2)	64 (0–3)
Septal fibrosis	3 (0–1)	1 (0–1)	61 (0–3)	92 (0–3)	75 (0–4)	99 (0–4)	61 (0–3)	98 (0–4)	67 (0–4)	98 (0–4)
Alveolar proteinosis	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	18 (0–2)	25 (0–3)	3 (0–1)	4 (0–2)	70 (0–4)	96 (0–4)
Bronchiolar-alveolar metaplasia	0 (0–0)	0 (0–0)	2 (0–1)	15 (0–1)	25 (0–2)	66 (0–4)	6 (0–2)	32 (0–3)	37 (0–2)	84 (0–4)
Focal fibrosis with epithelial hyperplasia	0 (0–0)	0 (0–0)	0 (0–0)	6 (0–2)	2 (0–2)	25 (0–3)	0 (0–0)	6 (0–3)	0 (0–0)	22 (0–3)
Squamous metaplasia	0 (0–0)	0 (0–0)	2 (0–1)	1 (0–1)	2 (0–3)	3 (0–2)	0 (0–0)	1 (0–1)	0 (0–0)	8 (0–3)
Number of rats	32	86	44	71	44	71	33	81	30	85

[a] Percentage (and range of severity scores) of male rats dying, euthanized, or sacrificed before 18 months of exposure that had each nonneoplastic lung lesion.

[b] Percentage (and range of severity scores) of male rats dying, euthanized, or sacrificed after 18 months of exposure that had each nonneoplastic lung lesion.

increased septal fibrosis correlated with an increased interstitial aggregation of particle-containing macrophages. The severity of septal fibrosis increased with exposure concentration and duration of exposure.

Alveolar proteinosis. Alveolar proteinosis was characterized by an accumulation of eosinophilic, granular or refractile, homogeneous, acellular material in the alveoli. DE soot or CB particles were present within this material. Alveolar proteinosis, which was first observed in one HDE rat at 6 months, was present in most HDE and HCB rats by 12 months and occurred later in LDE and LCB rats. The severity of the lesion increased with exposure concentration and time and, at all times after 12 months, was more severe in females than in males.

At approximately 18 months of exposure, there was an increase in the amount of DE soot or CB particles that was associated with alveolar proteinosis or cellular debris in alveoli. Most of the debris appeared to be the remains of macrophages. From 18 months on, the proportion of alveolar space that contained DE soot or CB particles not within viable macrophages was greater for the HDE than the HCB group. Also, the CB particles tended to be localized more to the centriacinar region than DE soot, leaving correspondingly more particle-free alveoli in CB- than in DE-exposed rats.

Bronchiolar-alveolar metaplasia. Bronchiolar-alveolar metaplasia denotes the presence of columnar, ciliated cells lining alveolar ducts and adjacent alveoli. Bronchiolar-alveolar metaplasia was first observed at approximately 12 months in HDE and HCB rats. This lesion was present in most exposed rats by 18 months, and the severity increased with exposure concentration and time. The severity, at any specified time interval after 12 months, was greater in females than in males.

Focal fibrosis with epithelial hyperplasia. Focal fibrosis with alveolar epithelial hyperplasia consisted of a well-circumscribed, nodular focus of fibrosis that obliterated the pulmonary architecture. The center of the lesion was composed of dense collagen bundles and small alveolar structures lined by cuboidal epithelium. A thick rim of hyperplastic, sometimes papillary, epithelium surrounded the periphery of the scirrhous nodule. Numerous neutrophils were associated with this hyperplastic epithelium. This lesion occurred late in the study. It was first observed at 16 months in an HCB female and at 17 months in an LDE female. The first focal fibrosis with epithelial hyperplasia was observed in the other gender and exposure groups at 18 to 23 months of exposure. The severity increased with exposure concentration and time.

Squamous metaplasia. Squamous metaplasia of alveolar epithelium was first observed at approximately 18 months in an LCB male and in an HCB male. Squamous metaplasia was diagnosed in the other gender and exposure groups at 18 to 23 months of exposure. Squamous metaplasia occurred infrequently compared to the more common lesions listed above.

Squamous cysts. Squamous cysts were lined by well-differentiated, stratified squamous epithelium with a central keratin accumulation. These lesions were sharply demarcated, except in those areas in which metaplasia extended into adjacent alveoli. The squamous epithelium had few mitotic figures, and dysplasia was absent. The lesion appeared to grow by keratin accumulation and peripheral extension of metaplasia into adjacent alveolar spaces.

The first squamous cyst was observed in an HDE female at approximately 19 months. The first lesion was observed in the other gender and exposure groups from 20 months to the final sacrifice. The prevalence of squamous cysts in each exposure group and gender is shown in Table 7. The prevalence of squamous cysts was significantly higher at the high exposure levels than at the low exposure levels. The number of female rats with squamous cysts (37) was greater than the number of male rats with cysts (10). However, the cysts were observed in 20 of the female rats after 24 months of exposure.

Logistic regression modeling for nonneoplastic lesions. For most of the nonneoplastic lesions, similar results were obtained for both genders. The probability of a higher score was statistically significant for the higher exposure concentration and for exposure to DE. This is shown in Table 8 by the odds ratios and the 95% confidence intervals that do not include 1.0. The only lesions that were not more severe in DE-exposed than in CB-exposed rats were focal fibrosis and squamous metaplasia. For focal fibrosis, the difference between exposure to DE and CB was marginally significant with DE causing a greater response in females ($p = 0.05$) and a lesser response in males ($p = 0.05$). For squamous metaplasia, the difference between exposure to DE and CB

TABLE 7

Prevalences of Female and Male Rats with One or More Squamous Cysts by Time Interval from the Start of Exposure

	18–24 Months[a]		>24 Months	
Group	F	M	F	M
Control	0/56[b]	0/77	0/35	0/9
LCB	1/54	0/72	7/36	1/1
HCB	5/69	4/74	8/18	0/0
LDE	1/53	0/73	2/29	1/9
HDE	10/68	4/84	3/22	0/1

[a] Squamous cysts were not observed in rats that died before 18 months of exposure.

[b] Number of rats having one or more squamous cysts/number of rats dying in the time interval.

TABLE 8
Odds Ratios for Nonneoplastic Lesions in Female and Male Rats

Nonneoplastic lesion factors	Odds ratio[a] (95% CI) Females	Males
Alveolar macrophage hyperplasia		
High to low concentration	38 (22, 66)	29 (17, 51)
DE to CB	5.4 (3.6, 8.1)	4.0 (2.6, 6.1)
Alveolar epithelial hyperplasia		
High to low concentration	15 (9.4, 23)	13 (8.6, 21)
DE to CB	1.6 (1.1, 2.3)	4.0 (2.7, 5.9)
Chronic-active inflammation		
High to low concentration	3.4 (2.4, 5.0)	6.2 (3.9, 9.8)
DE to CB	1.7 (1.2, 2.4)	2.4 (1.5, 3.8)
Septal fibrosis		
High to low concentration	10 (6.4, 16)	9.6 (6.2, 15)
DE to CB	1.7 (1.2, 2.5)	3.4 (2.3, 5.0)
Alveolar proteinosis		
High to low concentration	210 (110, 410)	860 (250, 3000)
DE to CB	29 (17, 48)	64 (30, 140)
Bronchiolar-alveolar metaplasia		
High to low concentration	20 (12, 31)	24 (14, 41)
DE to CB	5.0 (3.4, 7.3)	2.1 (1.4, 3.3)
Focal fibrosis with epithelial hyperplasia		
High to low concentration	3.8 (2.3, 6.2)	12 (5.0, 30)
DE to CB	1.6 (1.0, 2.5)	0.49 (0.24, 1.0)
Squamous metaplasia		
High to low concentration	5.4 (2.6, 11)	4.4 (1.1, 17)
DE to CB	0.86 (0.46, 1.6)	1.2 (0.37, 3.9)

[a] The odds of the higher exposure concentration to the lower exposure concentration and the odds of DE exposure to CB exposure. The two odds ratios were estimated at the same time in a polychotomous logistic regression model while also having a linear term for time on study. When the 95% confidence interval (CI) does not include 1, then the results for the exposure concentrations or exposure materials are significantly different from each other at $p = 0.05$.

was not significant in females ($p = 0.62$) or males ($p = 0.75$).

The coefficients of the logistic regression model for squamous cysts are shown in Table 9. The coefficients of slope for the response to either CB or DE were significantly increased compared to controls for females ($p < 0.001$) and for males ($p < 0.001$). There were no significant differences between the coefficients of slopes for the DE or CB responses for females ($p = 0.7$) or males ($p = 0.2$).

Neoplastic lung lesions. All primary lung neoplasms appeared to arise from the parenchyma; none appeared to arise from the conducting airways. Some smaller adenomas could be seen to arise from the parenchyma adjacent to the proximal alveolar ducts, the area where alveolar epithelial hyperplasia was most commonly located. Three key features differentiated the adenomas from foci of hyperplasia: (1) adenomas exhibited uniform, regular cells causing distortion of the alveolar architecture; (2) adenomas were unitized, discrete structures; and (3) adenomas exhibited an expansive growth that sometimes caused compression of the surrounding lung. The adenocarcinomas were differentiated from adenomas by their cellular anaplasia, invasion, in some cases, of lymphatics, blood vessels, or bronchial walls, and metastasis to regional lymph nodes.

There was evidence that the pathogenesis of adenomas and adenocarcinomas formed a continuum which began with alveolar epithelial hyperplasia. In some cases, the adenomas and adenocarcinomas were surrounded by epithelial hyperplasia. Several adenocarcinomas appeared to arise within adenomas, and the morphological features of the neoplasms suggested that they arose from alveolar epithelium. Also, the prevalence of alveolar epithelial hyperplasia increased much earlier than the prevalence of adenocarcinomas. The location of the squamous cell carcinomas and the observation of squamous metaplasia in the alveolar region suggested that the squamous cell carcinomas arose from alveolar epithelium, but a benign squamous neoplasm linking metaplasia and carcinoma was not evident. The first squamous cell carcinomas occurred before squamous metaplasia of alveolar epithelium or squamous cysts were observed.

The occurrence of the different types of lung neoplasms is summarized in Table 10, which lists the total number of rats examined for neoplasms, the number of rats with neoplasms, the number of each type of neoplasm observed, and the number of rats having each type of neoplasm by gender and treatment group. Three male C rats had primary lung neoplasms; one each had an adenoma, an adenocarcinoma, and a squamous cell carcinoma. No lung neoplasms were found in C females. No neoplasm type or morphology was distinctive for CB or DE exposure. Adenomas, adenocarcinomas, and squamous cell carcinomas occurred in all treatment groups of both genders, except that there were no squamous cell carcinomas in LCB rats and no adenomas in HCB male rats. Two adenosquamous carcinomas occurred in HCB rats (one female, one male), and one occurred in a female HDE rat. Most neoplasms were adenomas and ade-

TABLE 9
Estimated Coefficients of Logistic Regression Model for Squamous Cysts for Each Gender

Factors	Females Coefficient (SE)	Males Coefficient (SE)
Intercept	−10.2 (2.1)	−30.9 (7.2)
Time on study (days)	0.00878 (0.00291)	0.03549 (0.0096)
Diesel (mg · day/m^3)	0.00051 (0.00013)	0.00096 (0.00032)
Carbon black (mg · day/m^3)	0.00055 (0.00012)	0.00122 (0.00033)

TABLE 10
Numbers of Different Types of Lung Neoplasms Observed, and Numbers of Rats with Each Type of Neoplasm[a]

	C			LCB			HCB			LDE			HDE		
	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total	Female	Male	Total
Number of susceptible rats[b]	105	109	214	107	106	213	105	106	211	105	105	210	106	106	212
Total rats with neoplasms	0	3	3	8	2	10	28	4	32	8	5	13	29	9	38
Tumor type															
Adenoma															
Number of neoplasms	0	1	1	2	1	3	17	0	17	6	2	8	22	5	27
Rats with neoplasms	0	1	1	2	1	3	13	0	13	5	2	7	19	4	23
Adenocarcinoma															
Number of neoplasms	0	1	1	6	1	7	23	1	24	3	1	4	32	3	35
Rats with neoplasms	0	1	1	6	1	7	20	1	21	3	1	4	19	3	22
Squamous cell carcinoma															
Number of neoplasms	0	1	1	0	0	0	1	2	3	1	2	3	1	2	3
Rats with neoplasms	0	1	1	0	0	0	1	2	3	1	2	3	1	2	3
Adenosquamous carcinoma															
Number of neoplasms	0	0	0	0	0	0	1	1	2	0	0	0	1	0	1
Rats with neoplasms	0	0	0	0	0	0	1	1	2	0	0	0	1	0	1
Other															
Number of neoplasms	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Rats with neoplasms	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0

[a] Several individual rats had multiple types of tumors and/or multiple tumors of a single type; thus, these rats are counted more than once in this table.
[b] Values include all rats examined by gross necropsy and microscopy except rats sacrificed at 3, 6, and 12 months. The first lung neoplasm was observed between 12 and 18 months of exposure; thus, all rats dying spontaneously, or euthanized in moribund condition, plus those sacrificed at 18 months or later were considered at risk for expression of lung neoplasia. The total number of rats examined, including those sacrificed at 3, 6, and 12 months, is listed in Table 1.

nocarcinomas, which occurred far more often in females than in males. This gender difference was most striking at the higher exposure levels of DE and CB. In contrast, slightly more squamous cell carcinomas occurred in males than in females.

Only single lung neoplasms were observed in C rats, but the data in Table 12 demonstrate that multiple neoplasms were observed in rats in the exposed groups, particularly in females exposed to the high concentrations of CB or DE. A total of 117 neoplasms, 47 benign and 70 malignant, were observed in 73 neoplasm-bearing female rats. A total of 24 neoplasms, 9 benign and 15 malignant, were observed in 23 neoplasm-bearing male rats. One of the benign neoplasms and 2 of the malignant neoplasms occurred in control male rats. In female rats, the multiplicity of neoplasms was dose-related, and multiple neoplasms were slightly more prevalent in the HDE- than in the HCB-exposed group. Only 1 male HDE rat had more than 1 neoplasm. About 64 and 45% of the neoplasm-bearing HCB and HDE female rats, respectively, had single lung neoplasms; approximately 14 and 24%, respectively, had 3 or more neoplasms.

Lung neoplasms in DE- and CB-exposed rats appeared late during the exposure, as observed previously in DE-exposed rats (Mauderly *et al.*, 1987). The observation of neoplasms with time is illustrated in Fig. 3, in which the cumulative number of rats with at least one benign or malignant neoplasm is plotted versus time after initiating exposure. Lung neoplasms tended to accumulate earlier in the high concentration than in the low concentration groups for both exposure materials. For each concentration group and each material, neoplasms occurred in male rats before female rats. Before 650 days, one rat with a neoplasm was found in each of the male C, male LCB, female LCB, male LDE, and male HDE groups. By the same time, two rats with neoplasms were found in both the male and female HCB groups. Approximately one-half of the neoplasm-bearing female rats from all exposures were in the final sacrifice groups.

Only 11 primary lung neoplasms were considered nonincidental. Three of these occurred in the HDE females, four occurred in the HCB females, and one in an LCB female. Two nonincidental neoplasms occurred in the HDE males and one occurred in an LDE male. The data most closely representing traditional measures of incidence are listed in Table 11, which presents the numbers of rats of each gender in each group observed to have at least one lung neoplasm and the percentages that these rats represented of the numbers of rats considered at risk and examined for lung neoplasms. The numbers of rats considered at risk for the development and observation of lung neoplasms were all of the sacrificed, dying, or euthanized rats whose lungs were examined microscopically, except those sacrificed at 3, 6, and 12 months. Rats sacrificed at these times were not considered "at risk" because the first lung neoplasm was observed at approximately 15 months.

The percentages of female rats with neoplasms were not strikingly different between the LCB and LDE groups or between the HCB and HDE groups, regardless of whether

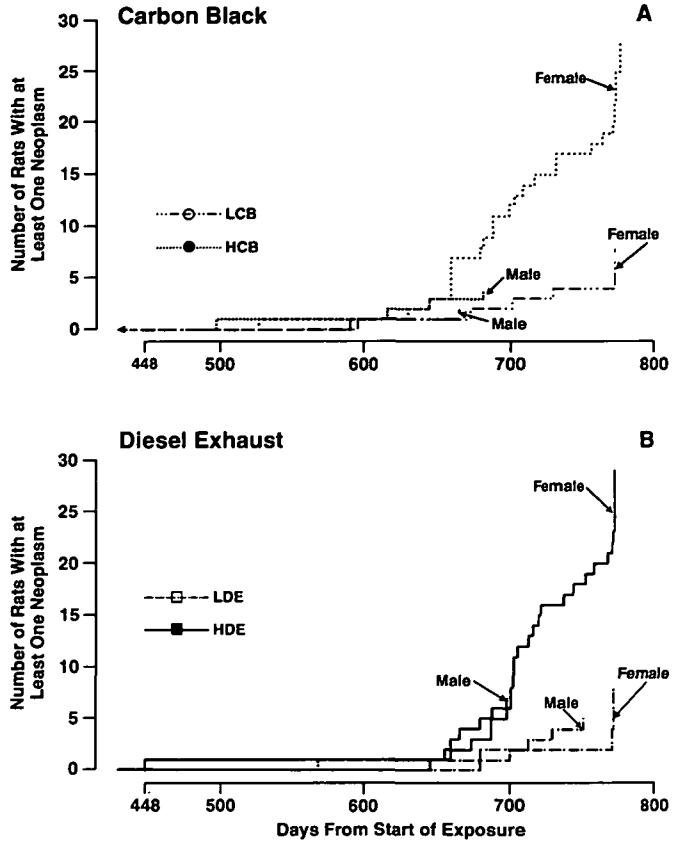

FIG. 3. The cumulative number of rats in each exposure group (A, carbon black; B, diesel exhaust) observed to have at least one malignant or benign lung neoplasm is illustrated.

only malignant or both malignant and benign neoplasms were considered. The percentages of exposed male rats with neoplasms were lower than the percentages of exposed female rats with neoplasms. The percentages of male rats with malignant or both malignant and benign neoplasms were small, and it is difficult to see differences between groups.

Figure 4 illustrates the prevalences of rats having at least one malignant or benign lung neoplasm. The figure illustrates clearly that the prevalences of lung neoplasms were concentration-related, higher in females than in males, and very similar for the DE and CB groups at each concentration. The prevalence of lung neoplasms was highest for females dying during the last interval, after exposures were terminated. Although DE- and CB-exposed males had lower neoplasm prevalences during the last two intervals, there were few male survivors during these intervals compared to the number of female survivors. No HCB males lived to the last interval. Only two HDE males lived to the last interval, and neither had lung neoplasms. The much larger error bars for the neoplasm prevalence curves for males reflect this difference in survival.

Statistical comparisons of the lung neoplastic responses among treatment groups were done using logistic regression modeling, as described under Materials and Methods. The appropriateness of the logistic regression model was predicated on the assumption that the lung neoplasms, as a group, were incidental to the death of the rats. This means that the average mortality rate for rats with neoplasms was the same as the mortality rate for rats without neoplasms. Because the study design included rats randomly chosen for interim sacrifices from the living animals and these rats were evaluated for the presence of lung neoplasms, the assumption that the neoplasms were incidental could be tested. This was done by comparing, in a logistic regression model, the prevalence of tumors in the randomly selected interim-sacrifice rats with the prevalence in those dying spontaneously or that were sacrificed as moribund. A model for each gender with terms like those in Table 12 for the interim-sacrifice rats and the rats dying spontaneously showed that there was no significant difference for females ($p = 0.7$) or males ($p = 0.2$).

The coefficients of the logistic regression model are shown in Table 12. The coefficients of slope for the neoplastic response to CB were not significantly different from the coefficients of slope for the response to DE for either females ($p = 0.62$) or males ($p = 0.23$), as determined by a likelihood ratio test. The coefficients of slope for the neoplastic response to either CB or DE were significantly different from controls for both genders ($p < 0.001$ for females and $p = 0.03$ for males).

DISCUSSION

The nonneoplastic responses to DE and CB were qualitatively similar. The magnitudes of most responses to both materials were generally related to particle exposure concentrations, with some parameters reflecting the greater lung burdens of DE soot than of CB. The life-span shortening, body weight reductions, and increases in lung weight of both females and males were greater in the HCB groups than in the LDE groups, which had similar lung burdens of particles. The increase in lung weight above that of the C group reflected the inflammatory, proliferative, and fibrotic lesions. The accumulated mass of particles contributed a negligible fraction of the increase in weight; for example, the 90.6-mg lung burden of the HDE males at 23 months was only 2.3% of the 3.99-g increase in lung weight above that of the C group. In both females and males, the incidences and severities of histopathological scores tended to be related to the lung burdens of particles, with responses of the HDE group greater than those of the HCB group. However, both the incidences and severities tended to be slightly higher for the HCB group than for the LDE group, which had a similar lung burden. The above findings indicate that overall, the nature and magnitudes of nonneoplastic re-

TABLE 11
Summary of Numbers and Percentages of Rats Examined for Lung Neoplasms with One or More Neoplasms[a]

Group	Gender	Number of susceptible rats examined for neoplasms[b]	Rats with malignant neoplasms		Rats with malignant or benign neoplasms	
			Number	Percentage of susceptible rats	Number	Percentage of susceptible rats
C	F	105	0	0	0	0
	M	109	2	1.8	3	2.8
	F + M	214	2	0.9	3	1.4
LCB	F	107	7	6.5	8	7.5
	M	106	1	0.9	2	1.9
	F + M	213	8	3.8	10	4.7
HCB	F	105	21	20.0	28	26.7
	M	106	4	3.8	4	3.8
	F + M	211	25	11.8	32	15.2
LDE	F	105	4	3.8	8	7.6
	M	105	3	2.9	5	4.8
	F + M	210	7	3.3	13	6.2
HDE	F	106	21	19.8	29	27.4
	M	106	5	4.7	9	8.5
	F + M	212	26	12.3	38	17.9

[a] Each rat with one or more neoplasms was counted only once in each neoplasm category.
[b] Values include all rats examined by gross necropsy and microscopy except rats sacrificed at 3, 6, and 12 months. The first lung neoplasm was observed between 12 and 18 months of exposure; thus, all rats dying spontaneously, or euthanized in moribund condition, plus those sacrificed at 18 months or later were considered at risk for expression of lung neoplasia. The total number of rats examined, including those sacrificed at 3, 6, and 12 months, is listed in Table 1.

sponses to DE and CB were very similar at similar particle exposure concentrations.

Although this study did not directly test the importance of soot-associated mutagenic organic compounds in the lung cancer response of rats heavily exposed to DE, it provided an indirect evaluation. The CB was not devoid of organic matter, but considering both the mass fraction of solvent-extractable matter and its mutagenicity, the CB mutagenicity per unit of particle mass was 2.6 or 5.5×10^{-3} (depending on bacterial strain) that of the DE soot, when measured using extraction methods and mutagenicity assays commonly applied to DE soot. We do not have data concerning the bioavailability of the mutagenic fraction or the kinetics of release of the mutagenic fraction in the rat lung. Organic residues adhere tenaciously to CB, and exhaustive, high-temperature extractions are required to fully remove them (IARC, 1983). Locati *et al.* (1979) extracted five CBs, all furnace blacks, for 350 hr with benzene using the soxhlet method and found that at least 150 hr were required to extract 95% of the organic fraction, which ranged from 0.025 to 0.142% of the CB mass. Neal *et al.* (1962) found no elution of polycyclic hydrocarbons from furnace black or channel black after 180 hr of incubation with agitation at body temperature in gastric and intestinal fluid simulants, citric acid mixtures, or other body fluid and foodstuff media. Although the extraction used in the present study might not have removed all organic residues from the CB, it seems unlikely that the remaining material would have dissociated under physiological conditions in the lung.

This study clearly demonstrated that the carcinogenic responses to DE and CB exposure were very similar. Logistic regression modeling did not demonstrate significant differences between the neoplastic responses to CB and DE for either gender. The numbers of CB-exposed rats with neoplasms tended to accumulate slightly earlier than those of DE-exposed rats. The crude incidences and the multiplicity of neoplasms tended to be slightly higher among DE- than among CB-exposed rats, but the differences were small, and the crude incidence would have been suppressed in groups such as the LCB and HCB males with shortened survival. Finally, the lung burdens of DE soot were higher than those of CB.

Exposures to CB and DE caused significant, exposure concentration-related increases, of similar magnitudes, in the incidences and prevalences of the same types of malignant and benign lung neoplasms in the female rats. In the logistic regression models, the errors for the estimates of the slopes were large, particularly for the males. This occurred because of the much shorter life span of the males. The prevalence of lung neoplasms increased rapidly near the

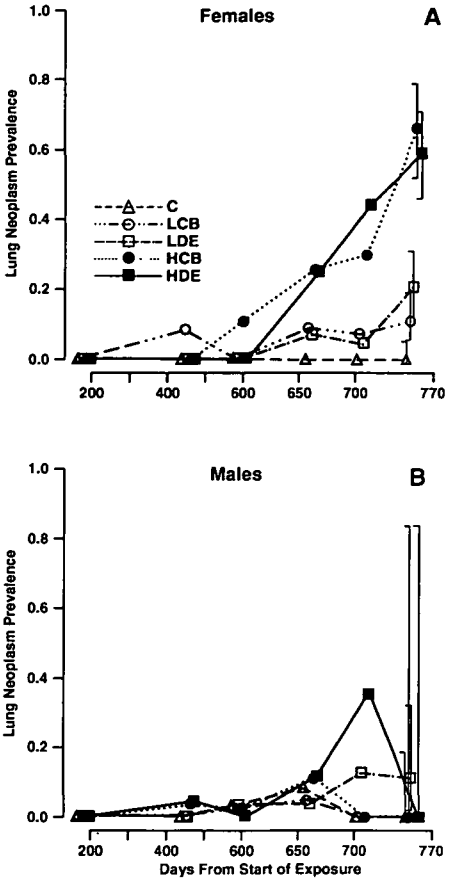

FIG. 4. The prevalence of rats observed to have malignant or benign lung neoplasms during various intervals after 200 days of exposure is illustrated. Prevalences are shown for females (A) and males (B). The data points shown are centered on the intervals representing <12 months, 12 to 18 months, 18 to 21 months, 21 to 22.5 months, 22.5 to 24 months, and >24 months (after end of exposure). The prevalences represent the fractions of rats sacrificed, dying, or euthanized during each interval which had one or more malignant or benign lung neoplasm. The error bars represent the SE of the estimate of prevalence, based on the number of rats observed during the last interval. Error bars for other intervals are omitted for clarity.

end of the life span of the females, at a time when most of the males had died from other causes. Furthermore, the absence of lung tumors in control females may have increased the estimated slopes for the females. These factors make the neoplastic response in males difficult to interpret. Thus, it is not completely clear whether there were true differences between the responses of males and females or if there were differences in the responses of the males to CB and DE. The logistic regression is a descriptive model, and for these types of exposures and results there are no mechanistic models that one might use to more confidently interpret the results.

Overall, CB was not significantly less carcinogenic than DE in this study. Based only on this comparison, the organic fraction of DE did not appear to play an important role in the carcinogenicity of DE observed in this study. This study did not prove that the soot-associated organic fraction played no role in carcinogenesis. However, if the organic fraction of DE soot played any role in the subtle differences between the expressions of lung neoplasia in the DE- and CB-exposed groups, the degree of its influence must have been small in comparison to the three orders of magnitude difference between the mutagenic organic contents of CB and DE soot.

The results of this study argue against extrapolating the data from the carcinogenicity in rats exposed heavily to DE to predict carcinogenicity in humans exposed at lower rates on the basis of chemical carcinogenesis. Although it is possible that soot-associated organic compounds contribute to lung cancer in humans, it does not appear reasonable to use the delivered dose of soot-associated organic compounds as the comparative dose term for extrapolation of carcinogenic risk from rats to humans. If it is assumed that soot-associated organic compounds are important in human lung cancer risk from DE exposure, then the results of this study raise questions about the validity of extrapolating from rat results to human risk regardless of the comparative dose term used. Unit risks of similar magnitude have been calculated for lifetime exposures of rats and humans using exposure concentration as the comparative dose term for linking bioassay and epidemiological data (Mauderly, 1992). The similarity of these estimates lends the greatest confidence to the usefulness of extrapolating the rat results to humans, but this confidence is eroded by the weakness of the exposure data for humans and the uncertainty about the similarity of the pathogenesis of the cancers in rats and humans.

The present results could be taken to suggest concern for the potential human lung cancer risk from inhaled CB or other fine carbon particles. Good dose–response information from rats exposed chronically by inhalation to CB at lower rates more relevant to human exposures is not available. Published epidemiological data (Smith and Musch, 1982) suggest that the higher time-weighted average exposures of workers in the CB industry are under 0.5 mg/m^3, and that elevated rates of lung cancer have not been associated with occupational exposures to CB. Considering that

TABLE 12
Estimated Coefficients of Logistic Regression Model for Lung Neoplasms for Each Gender

	Coefficient (SE)	
Factors	Females	Males
Intercept	−14.4 (2.1)	−9.7 (2.3)
Time on study (days)	0.01434 (0.00279)	0.00931 (0.00341)
Diesel (mg · day/m^3)	0.00085 (0.00012)	0.00038 (0.00015)
Carbon black (mg · day/m^3)	0.0081 (0.00011)	0.00020 (0.00018)

rats develop lung tumors when heavily exposed to a wide range of particles for which human lung cancer risk is uncertain or negative (Mauderly, 1994), the results of the present study should not raise concern for human lung cancer risk from current occupational exposures to CB.

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