5. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

The objective of this chapter is to review and evaluate potential health effects other than cancer associated with inhalation exposure to diesel exhaust (DE). Data have been obtained from diverse human, laboratory animal, and in vitro test systems. The human studies comprise both occupational and human experimental exposures, the former consisting of exposure to DE in the occupational environment, and the latter consisting of exposure to diluted DE or diesel particulate matter (DPM) under controlled conditions. The laboratory animal studies consist of both acute and chronic exposures of laboratory animals to DE or DPM. Diverse in vitro test systems composed of human and laboratory animal cells treated with DPM or components of DPM have also been used to investigate the effects of DPM at the cellular and molecular levels. DPM mass (mg/m^3) has been used as a measure of DE exposure in human and experimental studies. The noncancer health effects of DPM have been reviewed previously by the Health Effects Institute (HEI, 1995) and in the Air Quality for Particulate Matter Criteria Document (U.S. EPA, 1996). The noncancer health effects attributable to ambient particulate matter (PM), which is composed in part of DPM, as well as the potential mechanisms underlying these effects have also been previously reviewed in the Air Quality for Particulate Matter Criteria Document (U.S. EPA, 1996, also see chapter 6.2).

5.1. HEALTH EFFECTS OF WHOLE DIESEL EXHAUST

5.1.1. Human Studies

5.1.1.1. *Short-Term Exposures*

In a controlled human study, Rudell et al. (1990, 1994) exposed eight healthy subjects in an exposure chamber to diluted exhaust from a diesel engine for 1 h, with intermittent exercise. Dilution of the diesel exhaust was controlled to provide a median NO_2 level of approximately 1.6 ppm. Median particle number was $4.3 \times 10^6/cm^3$, and median levels of NO and CO were 3.7 and 27 ppm, respectively (particle size and mass concentration were not provided). There were no effects on spirometry or on closing volume using nitrogen washout. Five of eight subjects experienced unpleasant smell, eye irritation, and nasal irritation during exposure. Brochoalveolar lavage (BAL) was preformed 18 hours after exposure and was compared with a control BAL performed 3 weeks prior to exposure. There was no control air exposure. Small but statistically significant reductions were seen in BAL mast cells, AM phagocytosis of opsonized yeast particles, and lymphocyte CD4/CD8 ratios. A small increase in recovery of polymorphonuclear cells (PMNs) was also observed. These findings suggest that diesel exhaust may induce mild airway inflammation in the absence of spirometric changes. This study provides an intriguing glimpse of the effect of diesel exhaust exposure in humans, but only one exposure level was used, the number

of subjects was low, and a limited range of endpoints was reported, so the data are inadequate to generalize about the human response. To date, no well-controlled chamber study has been conducted using methodologies for assessing subtle lung inflammatory reactions.

Rudell et al. (1996) exposed volunteers to diesel exhaust for 1 h in an exposure chamber. Light work on a bicycle ergometer was performed during exposure. Exposures included either diesel exhaust or exhaust with particle numbers reduced 46% by a particle trap. The engine used was a new Volvo model 1990, a six-cylinder direct-injection turbocharged diesel with an intercooler, which was run at a steady speed of 900 rpm during the exposures. Comparison of this study with others was difficult because neither exhaust dilution ratios nor particle concentrations were reported. Carbon monoxide concentrations of 27-30 ppm and NO of 2.6-2.7 ppm, however, suggested DPM concentrations may have equaled several mg/m^3. The most prominent symptoms during exposure were irritation of the eyes and nose and an unpleasant smell. Both airway resistance and specific airway resistance increased significantly during the exposures. Despite the 46% reduction in particle numbers by the trap, effects on symptoms and lung function were not significantly attenuated.

Kahn et al. (1988) reported the occurrence of 13 cases of acute overexposure to diesel exhaust among Utah and Colorado coal miners. Twelve miners had symptoms of mucous membrane irritation, headache, and lightheadedness. Eight individuals reported nausea; four reported a sensation of unreality; four reported heartburn; three reported weakness, numbness, and tingling in their extremities; three reported vomiting; two reported chest tightness; and two others reported wheezing. Each miner lost time from work because of these symptoms, which resolved within 24 to 48 h. No air monitoring data were presented; poor work practices were described as the predisposing conditions for overexposure.

El Batawi and Noweir (1966) reported that among 161 workers from two garages where diesel-powered buses were serviced and repaired, 42% complained of eye irritation, 37% of headaches, 30% of dizziness, 19% of throat irritation, and 11% of cough and phlegm. Ranges of mean concentrations of diesel exhaust components in the two diesel bus garages were as follows: 0.4 to 1.4 ppm NO_2, 0.13 to 0.81 ppm SO_2, 0.6 to 44.1 ppm aldehydes, and 1.34 to 4.51 mg/m^3 of DPM; the highest concentrations were obtained close to the exhaust systems of the buses.

Eye irritation was reported by Battigelli (1965) in six subjects after 40 s of chamber exposure to diluted diesel exhaust containing 4.2 ppm NO_2, 1 ppm SO_2, 55 ppm CO, 3.2 ppm total hydrocarbons, and 1 to 2 ppm total aldehydes; after 3 min and 20 s of exposure to diluted diesel exhaust containing 2.8 ppm NO_2, 0.5 ppm SO_2, 30 ppm CO, 2.5 ppm total hydrocarbons, and <1 to 2 ppm total aldehydes; and after 6 min of exposure to diluted diesel exhaust containing 1.3 ppm NO_2, 0.2 ppm SO_2, <20 ppm CO, <2.0 ppm total hydrocarbons, and <1.0 ppm total aldehydes. The concentration of DPM was not reported.

Katz et al. (1960) described the experience of 14 chemists and their assistants monitoring the environment of a train tunnel used by diesel-powered locomotives. Although workers complained on three occasions of minor eye and throat irritation, no correlation was established with concentrations of any particular component of diesel exhaust.

The role of antioxidant defenses in protecting against acute diesel exhaust exposure has been studied. Blomberg et al. (1998) investigated changes in the antioxidant defense network within the respiratory tract lining fluids of human subjects following diesel exhaust exposure. Fifteen healthy, nonsmoking, asymptomatic subjects were exposed to filtered air or diesel exhaust (DPM 300 mg/m^3) for 1 h on two separate occasions at least 3 weeks apart. Nasal lavage fluid and blood samples were collected prior to, immediately after, and 5 ½ h post exposure. Bronchoscopy was performed 6 h after the end of diesel exhaust exposure. Nasal lavage ascorbic acid concentration increased tenfold during diesel exhaust exposure, but returned to basal levels 5.5 h postexposure. Diesel exhaust had no significant effects on nasal lavage uric acid or GSH concentrations, and did not affect plasma, bronchial wash, or bronchoalveolar lavage antioxidant concentrations, nor malondialdehyde or protein carbonyl concentrations. The authors concluded that the physiological response to acute diesel exhaust exposure is an acute increase in the level of ascorbic acid in the nasal cavity, which appears to be sufficient to prevent further oxidant stress in the respiratory tract of healthy individuals.

5.1.1.1.1. ***Diesel exhaust odor***. The odor of diesel exhaust is considered by most people to be objectionable; at high intensities, it may produce sufficient physiological and psychological effects to warrant concern for public health. The intensity of the odor of diesel exhaust is an exponential function of its concentration such that a tenfold change in the concentration will alter the intensity of the odor by one unit. Two human panel rating scales have been used to measure diesel exhaust odor intensity. In the first (Turk, 1967), combinations of odorous materials were selected to simulate diesel exhaust odor; a set of 12 mixtures, each having twice the concentration of that of the previous mixture, is the basis of the diesel odor intensity scale (D-scale). The second method is the TIA (total intensity of aroma) scale based on seven steps, ranging from 0 to 3, with 0 being undetectable, ½ very slight, and 1 slight and increasing in one-half units up to 3, strong (Odor Panel of the CRC-APRAC Program Group on Composition of Diesel Exhaust, 1979; Levins, 1981).

Surveys, utilizing volunteer panelists, have been taken to evaluate the general public's response to the odor of diesel exhaust. Hare and Springer (1971) and Hare et al. (1974) found that at a D rating of about 2 (TIA = 0.9, slight odor intensity), about 90% of the participants perceived the odor, and almost 60% found it objectionable. At a D rating of 3.2 (TIA = 1.2,

slight to moderate odor intensity), about 95% perceived the odor, and 75% objected to it, and, at a D rating of 5 (TIA = 1.8, almost moderate), about 95% objected to it.

Linnell and Scott (1962) reported odor threshold measurement in six subjects and found that the dilution factor needed to reach the threshold ranged from 140 to 475 for this small sample of people. At these dilutions, the concentrations of formaldehyde ranged from 0.012 to 0.088 ppm.

5.1.1.1.2. ***Pulmonary and respiratory effects***. Battigelli (1965) exposed 13 volunteers to three dilutions of diesel exhaust obtained from a one-cylinder, four-cycle, 7-hp diesel engine (fuel type unspecified) and found that 15-min to 1-h exposures had no significant effects on pulmonary resistance. Pulmonary resistance was measured by plethysmography utilizing the simultaneous recording of esophageal pressure and airflow determined by electrical differentiation of the volume signal from a spirometer. The concentrations of the constituents in the three diluted exhausts were 1.3, 2.8, and 6.2 ppm NO_2; 0.2, 0.5, and 1 ppm SO_2; <20, 30, and 55 ppm CO; and <1.0, <1 to 2, and 1 to 2 ppm total aldehydes, respectively. DPM concentrations were not reported.

A number of studies have evaluated changes in pulmonary function occurring over a workshift in workers occupationally exposed to diesel exhaust (specific time period not always reported but assumed to be 8 h). In a study of coal miners, Reger (1979) found that both forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) decreased by 0.05 L in 60 diesel-exposed miners, an amount not substantially different from reductions seen in non-diesel-exposed miners (0.02 and 0.04 L, respectively). Decrements in peak expiratory flow rates were similar between diesel and non-diesel exhaust-exposed miners. Miners with a history of smoking had an increased number of decrements over the shift than nonsmokers did. Although the monitoring data were not reported, the authors stated that there was no relationship between the low concentrations of measured respirable dust or NO_2 (personal samplers) when compared with shift changes for any lung function parameter measured for the diesel-exposed miners. This study is limited because results were preliminary (abstract) and there was incomplete information on the control subjects.

Ames et al. (1982) compared the pulmonary function of 60 coal miners exposed to diesel exhaust with that of a control group of 90 coal miners not exposed to diesel exhaust for evidence of acute respiratory effects associated with exposure to diesel exhaust. Changes over the workshift in FVC, FEV_1, and forced expiratory flow rate at 50% FVC (FEF_{50}) were the indices for acute respiratory effects. The environmental concentrations of the primary pollutants were 2.0 mg/m^3 respirable dust (<10 μm MMAD), 0.2 ppm NO_2, 12 ppm CO, and 0.3 ppm formaldehyde. The investigators reported a statistically significant decline in FVC and FEV_1 over the workshift in

both the diesel-exposed and comparison groups. Current smokers had greater decrements in FVC, FEV_1, and FEF_{50} than did ex-smokers and nonsmokers. There was a marked disparity between the ages and the time spent underground for the two study groups. Diesel-exposed miners were about 15 years younger and had worked underground for 15 fewer years (4.8 versus 20.7 years) than miners not exposed to diesel exhaust. The significance to the results of these differences between the populations is difficult to ascertain.

Except for the expected differences related to age, 120 underground iron ore miners exposed to diesel exhaust had no workshift changes in FVC and FEV_1 when compared with 120 matched surface miners (Jörgensen and Svensson, 1970). Both groups had equal numbers (30) of smokers and nonsmokers. The frequency of bronchitis was higher among underground workers, much higher among smokers than nonsmokers, and also higher among older than younger workers. The authors reported that the underground miners had exposures of 0.5 to 1.5 ppm NO_2 and between 3 and 9 mg/m^3 particulate matter, with 20% to 30% of the particles <5 μm MMAD. The majority of the particles were iron ore; quartz was 6% to 7% of the fraction <5 μm MMAD.

Gamble et al. (1979) measured preshift FEV_1 and FVC in 187 salt miners and obtained peak flow forced expiratory flow rates at 25%, 50%, and 75% of FVC (FEF_{25}, FEF_{50}, or FEF_{75}). Postshift pulmonary function values were determined from total lung capacity and flows at preshift percentages of FVC. The miners were exposed to mean NO_2 levels of 1.5 ppm and mean respirable particulate levels of 0.7 mg/m^3. No statistically significant changes were found between changes in pulmonary function and in NO_2 and respirable particles combined. Slopes of the regression of NO_2 and changes in FEV_1, FEF_{25}, FEF_{50}, and FEF_{75} were significantly different from zero. The authors concluded that these small reductions in pulmonary function were attributable to variations in NO_2 within each of the five salt mines that contributed to the cohort.

Gamble et al. (1987a) investigated the acute effects of diesel exhaust in 232 workers in four diesel bus garages using an acute respiratory questionnaire and before and after workshift spirometry. The prevalence of burning eyes, headaches, difficult or labored breathing, nausea, and wheeze experienced at work was higher in the diesel bus garage workers than in a comparison population of lead/acid battery workers who had not previously shown a statistically significant association of acute symptoms with acid exposure. Comparisons between the two groups were made without adjustment for age and smoking. There was no detectable association of exposure to NO_2 (0.23 ppm ± 0.24 S.D.) or inhalable (less than 10 μm MMAD) particles (0.24 mg/m^3 ± 0.26 S.D.) and acute reductions in FVC, FEV_1, peak flows, FEF_{50}, and FEF_{75}. Workers who had respiratory symptoms had slightly greater but statistically insignificant reductions in FEV_1 and FEF_{50}.

Ulfvarson et al. (1987) evaluated workshift changes in the pulmonary function of 17 bus garage workers, 25 crew members of two types of car ferries, and 37 workers on roll-on/roll-off ships. The latter group was exposed primarily to diesel exhaust; the first two groups were exposed to both gasoline and diesel exhaust. The diesel-only exposures that averaged 8 h consisted of 0.13 to 1.0 mg/m^3 particulate matter, 0.02 to 0.8 mg/m^3 (0.016 to 0.65 ppm) NO, 0.06 to 2.3 mg/m^3 (0.03 to 1.2 ppm) NO_2, 1.1 to 5.1 mg/m^3 (0.96 to 4.45 ppm) CO, and up to 0.5 mg/m^3 (0.4 ppm) formaldehyde. The largest decrement in pulmonary function was observed during a workshift following no exposure to diesel exhaust for 10 days. Forced vital capacity and FEV_1 were significantly reduced over the workshift (0.44 L and 0.30 L, $p<0.01$ and $p<0.001$, respectively). There was no difference between smokers and nonsmokers. Maximal midexpiratory flow, closing volume expressed as the percentage of expiratory vital capacity, and alveolar plateau gradient (phase 3) were not affected. Similar but less pronounced effects on FVC (−0.16 L) were found in a second, subsequent study of stevedores (n = 24) only following 5 days of no exposure to diesel truck exhaust. Pulmonary function returned to normal after 3 days without occupational exposure to diesel exhaust. No exposure-related correlation was found between the observed pulmonary effects and concentrations of NO, NO_2, CO, or formaldehyde; however, it was suggested that NO_2 adsorbed onto the diesel exhaust particles may have contributed to the overall dose of NO_2 to the lungs. In a related study, six workers (job category not defined) were placed in an exposure chamber and exposed to diluted diesel exhaust containing 0.6 mg/m^3 DPM and 3.9 mg/m^3 (2.1 ppm) NO_2. The exhaust was generated by a 6-cylinder, 2.38-L diesel engine, operated for 3 h and 40 min at constant speed, equivalent to 60 km/h, and at about one-half full engine load. No effect on pulmonary function was observed.

The relationship between traffic density and respiratory health in children has been examined in a series of studies in Holland in children attending schools located near major freeways. Cough, wheeze, runny nose, and doctor-diagnosed asthma were reported more often for children living within 100 m of freeways carrying between 80,000 and 150,000 vehicles per day (van Vliet et al., 1997). Separate counts for truck traffic indicated a range from 8,000 to 17,500 trucks per day. Truck traffic intensity and the concentration of black smoke, considered by the authors to be a proxy for DPM, measured in schools were found to be significantly associated with chronic respiratory symptoms, with the relationships being more pronounced in girls than in boys.

Brunekreef et al. (1997) measured lung function in children in six areas located near major motorways and assessed their exposure to traffic-related air pollution using separate traffic counts for automobiles and trucks. They also measured air pollution in the children's schools. While lung function was associated with truck traffic density, there was a lesser association with automobile traffic density. The association was stronger in those children living closest (300 m) to the

roadways. Lung function was also associated with concentration of black smoke, measured inside the schools. The associations were stronger in girls than in boys. The authors conclude that exposure to vehicular pollution, in particular DPM, may lead to reduced lung function in children living near major motorways.

In a follow-up study of traffic-related air pollution and its effect on the respiratory health of children living near roadways, Brunekreef et al. (2000) showed that the intensity of truck traffic was significantly associated with the prevalence of wheeze, phlegm, bronchitis, eye symptoms, and allergy to dust and pets. Associations with yearly averaged $PM_{2.5}$ and "soot" concentrations measured inside and outside the schools showed similar patterns. Truck traffic intensity was also significantly associated with a positive skin prick test or elevated IgE for outdoor allergens. There were no associations between traffic intensity or $PM_{2.5}$ and "soot" concentrations and lung function, bronchial responsiveness, and allergic reactions to indoor allergens. Further analysis of the data showed that the associations between traffic-related air pollution and symptoms were almost entirely related to children with bronchial hyperreactivity or sensitization to common allergens.

5.1.1.1.3. ***Immunological effects***. Salvi et al. (1999) exposed healthy human subjects to diluted diesel exhaust (DPM 300 $\mu g/m^3$) for 1 h with intermittent exercise. Although there were no changes in pulmonary function, there were significant increases in neutrophils and B lymphocytes as well as histamine and fibronectin in airway lavage fluid. Bronchial biopsies obtained 6 h after diesel exhaust exposure showed a significant increase in neutrophils, mast cells, and CD4+ and CD8+ T lymphocytes, along with upregulation of the endothelial adhesion molecules ICAM-1 and VCAM-1 and increases in the number of LFA-1+ in the bronchial tissue. Significant increases in neutrophils and platelets were observed in peripheral blood following exposure to diesel exhaust.

In a follow-up investigation of potential mechanisms underlying the DE-induced airway leukocyte infiltration, Salvi et al. (2000) exposed healthy human volunteers to diluted DE, on two separate occasions for 1 h each, in an exposure chamber. Fiber-optic bronchoscopy was performed 6 h after each exposure to obtain endobronchial biopsies and bronchial wash (BW) cells. These workers observed that DE exposure enhanced gene transcription of IL-8 in the bronchial tissue and BW cells and increased growth-regulated oncogene-α protein expression and IL-8 in the bronchial epithelium; there was also a trend toward an increase in IL-5 mRNA gene transcripts in the bronchial tissue.

In an attempt to evaluate the potential allergenic effects of DPM in humans, Diaz-Sanchez and associates carried out a series of clinical investigations. In the first of these (Diaz-Sanchez et al., 1994), healthy human volunteers were challenged by spraying either saline or 0.30 mg DPM

into their nostrils. This dose was considered equivalent to total exposure on 1-3 average days in Los Angeles, but could occur acutely in certain nonoccupational settings such as sitting at a busy bus stop or in an express tunnel. Enhanced IgE levels were noted in nasal lavage cells in as little as 24 h, with peak production observed 4 days after DPM challenge. The effects seemed to be somewhat isotype-specific, because in contrast to IgE results, DPM challenge had no effect on the levels of IgG, IgA, IgM, or albumin. The selective enhancement of local IgE production was demonstrated by a dramatic increase in IgE-secreting cells.

Although direct effects of DPM on B-cells have been demonstrated by in vitro studies, it was considered likely that other cells regulating the IgE response may also be affected. Cytokine production was therefore measured in nasal lavage cells from healthy human volunteers challenged with DPM (0 or 0.15 mg in 200 μL saline) sprayed into each nostril (Diaz-Sanchez et al., 1996). Before challenge with DPM, most subjects' nasal lavage cells had detectable levels of only interferon- , IL-2, and IL-13 *m*RNA. After challenge with DPM, the cells produced readily detectable levels of *m*RNA for IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and interferon- . In addition, all levels of cytokine *m*RNA were increased. Although the cells in the nasal lavage before and after challenge do not necessarily represent the same ones either in number or type, the broad increase in cytokine production was not simply the result of an increase in T cells recovered in the lavage fluid. On the basis of these findings, the authors concluded that the increase in nasal cytokine expression after exposure to DPM can be predicted to contribute to enhanced local IgE production and thus play a role in pollutant-induced airway disease.

The ability of DPM to act as an adjuvant to the ragweed allergen Amb a I was also examined by nasal provocation in ragweed-allergic subjects using 0.3 mg DPM, Amb a I, or both (Diaz-Sanchez et al., 1997). Although allergen and DPM each enhanced ragweed-specific IgE, DPM plus allergen promoted a 16-times greater antigen-specific IgE production. Nasal challenge with DPM also influenced cytokine production. Ragweed challenge resulted in a weak response, DPM challenge caused a strong but nonspecific response, and allergen plus DPM caused a significant increase in the expression of mRNA for TH0 and TH2-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13), with a pronounced inhibitory effect on IFN- gene expression. The author concluded that DPM can enhance B-cell differentiation and, by initiating and elevating IgE production, may be a factor in the increased incidence of allergic airway disease.

In a further extension of these studies, Diaz-Sanchez et al. (1999) examined the potential for DPM to lead to primary sensitization of humans by driving a de novo mucosal IgE response to a neoantigen, keyhole limpet hemocyanin (KLH). Ten atopic subjects were given an initial nasal immunization of KLH followed by two biweekly nasal challenges with KLH. Fifteen different atopic subjects were treated identically, except that DPM was administered 24 h before each KLH exposure. Intranasal administration of KLH alone led to the generation of an anti-KLH IgG and

IgA humoral response, which was detected in nasal fluid samples. No anti-KLH IgE was observed in any of these subjects. In contrast, when challenged with KLH preceded by DPM, 9 of the 15 subjects produced anti-KLH-specific IgE. KLH-specific IgG and IgA at levels similar to those seen with KLH alone were also detected. Subjects who received DPM and KLH had significantly increased IL-4, but not IFN-gamma, levels in nasal lavage fluid, whereas these levels were unchanged in subjects receiving KLH alone. These investigators concluded that DPM can function as a mucosal adjuvant to a de novo IgE response and may increase allergic sensitization.

5.1.1.1.4. ***Human cell culture studies*****.** The potential mechanisms by which DPM may act to cause allergenic effects has been examined in human cell culture studies. Takenaka et al. (1995) reported that DPM extracts enhanced IgE production from purified human B cells. Interleukin-4 plus monoclonal antibody-stimulated IgE production was enhanced 20% to 360% by the addition of DPM extracts over a period of 10-14 days. DPM extracts themselves did not induce IgE production or synergize with interleukin-4 alone to induce IgE from purified B cells, suggesting that the extracts were enhancing ongoing IgE production rather than inducing germline transcription or isotype switching. The authors concluded that enhancement of IgE production in the human airway resulting from the organic fraction of DPM may be an important factor in the increasing incidence of allergic airway disease.

Steerenberg et al. (1998) studied the effects of exposure to DPM on airway epithelial cells, the first line of defense against inhaled pollutants. Cells from a human bronchial cell line (BEAS-2B) were cultured in vitro and exposed to DPM (0.04-0.33 mg/mL) and the effects on IL-6 and IL-8 production were observed. Increases in IL-6 and IL-8 production compared to the nonexposed cells (11- and 4-fold, respectively) were found after 24 or 48 h exposure to DPM. This increase was lower (17- and 3.3-fold) compared to silica and higher compared to titanium dioxide, which showed no increase for either IL-6 or IL-8. The study was extended to observe the effects of DPM on inflammation-primed cells. BEAS-2B cells were exposed to TNF- followed by DPM. Additive effects on IL-6 and IL-8 production by BEAS-2B cells were found after TNF- priming and subsequent exposure to DPM only at a low dose of DPM and TNF- (0.05-0.2 ng/mL). The investigators concluded that BEAS-2B phagocytized DPM and produced an increased amount of IL-6 and IL-8, and that in TNF- -primed BEAS-2B cells DPM increased interleukin production only at low concentrations of DPM and TNF- .

Ohtoshi et al. (1998) studied the effect of suspended particulate matter (SPM), obtained from high-volume air samplers, and DPM on the production of IL-8 and granulocyte-colony stimulating factor (GM-CSF) by human airway epithelial cells in vitro. Nontoxic doses of DPMs stimulated production of IL-8 and GM-CSF by three kinds of human epithelial cells (nasal polyp-derived upper airway, normal bronchial, and transformed bronchial epithelial cells) in a

dose- and time-dependent fashion. SPM had a stimulatory effect on GM-CSF, but not on IL-8 production. The effects could be blocked with a protein synthesis inhibitor, suggesting that the process required de novo protein synthesis, and appeared to be due to an extractable component because neither charcoal nor graphite showed such stimulatory effects. The authors concluded that SPM and DPM, a component of SPM, may be important air pollutants in the activation of airway cells for the release of cytokines relevant to allergic airway inflammation.

The mechanisms underlying DPM-induced injury to airway cells were investigated in human bronchial epithelial cells (HBECs) in culture (Bayram et al., 1998a). HBECs from bronchial explants obtained at surgery were cultured and exposed to DPM (10-100 μg/mL) suspended in a serum-free supplemented medium (SF-medium) or to a SF-medium filtrate of DPM. The filtrate was obtained by incubating DPM (50 μg/mL) in SF-medium for 24 h. The effects of DPM and DPM filtrate on permeability, ciliary beat frequency (CBF), and release of inflammatory mediators were observed. DPM and filtered solution of DPM significantly increased the electrical resistance of the cultures but did not affect movement of bovine serum albumin across cell cultures. DPM and filtered DPM solution significantly attenuated the CBF of these cultures and significantly increased the release of IL-8. DPM also increased the release by these cultures of GM-CSF and soluble intercellular adhesion molecule-1 (sICAM-1). These authors also observed that activated charcoal was not able to induce changes in electrical resistance, attenuate CBF, and increase the release of inflammatory mediators from HBEC, and proposed that these effects were due most likely to the compounds adsorbed onto the DPM rather than the size of DPM. The authors concluded that exposure of airway cells to DPM may lead to functional changes and release of proinflammatory mediators and that these effects may influence the development of airway disease.

Bayram et al. (1998b) investigated the sensitivity of cultured airway cells from asthmatic patients to DPM. Incubation with DPM significantly attenuated the CBF in both the asthmatic and nonasthmatic bronchial epithelial cell cultures. Cultured airway cells from asthmatic patients constitutively released significantly greater amounts of IL-8, GM-CSF, and sICAM-1 than cell cultures from nonasthmatic subjects. Only cultures from asthmatic patients additionally released RANTES. The authors concluded that cultured airway cells from asthmatic subjects differ with regard to the amounts and types of proinflammatory mediators they can release and that the increased sensitivity of bronchial epithelial cells of asthmatic subjects to DPM may result in exacerbation of their disease symptoms.

Devalia et al. (1999) investigated the potential sensitivity of HBECs biopsied from atopic mild asthmatic patients and non-atopic nonasthmatic subjects to DPM. HBECs from asthmatic patients constitutively released significantly greater amounts of IL-8, GM-CSF, and sICAM-1 than HBECs from nonasthmatic subjects. RANTES was only released by HBECs of asthmatic

patients. Incubation of the asthmatic cultures with 10 µg/mL DPM significantly increased the release of IL-8, GM-CSF, and sICAM-1 after 24 h. In contrast, only higher concentrations (50-100 µg/mL DPM) significantly increased the release of IL-8 and GM-CSF from HBECs of nonasthmatics. The authors conclude that the increased sensitivity of the airways of asthmatics to DPM may be, at least in part, a consequence of greater constitutive and DPM-induced release of specific pro-inflammatory mediators from bronchial epithelial cells.

To elucidate the intracellular signal transduction pathway regulating IL-8 and RANTES production, Hashimoto et al. (2000) examined the role of p38 mitogen-activated protein (MAP) kinase in DPM-induced IL-8 and RANTES production by HBECs. They also examined the effect of a thiol-reducing agent, N-acetylcysteine (NAC), on DPM-induced p38 MAP kinase activation and cytokine production. The authors conclude that p38 MAP kinase plays an important role in the DPM-activated signaling pathway that regulates IL-8 and RANTES production by HBECs and that the cellular redox state is critical for DPM-induced p38 MAP kinase activation leading to IL-8 and RANTES production.

Boland et al. (1999) compared the biological effects of carbon black and DPM collected from catalyst- and noncatalyst-equipped diesel vehicles in cultures of both human bronchial epithelial cells and human nasal epithelial cells. Transmission electron microscopy indicated that DPM was phagocytosed by epithelial cells and translocated through the epithelial cell sheet. The time and dose dependency of phagocytosis and its nonspecificity for different particles (DPM, carbon black, and latex particles) were established by flow cytometry. DPM also induced a time-dependent increase in interleukin-8, GM-CSF, and interleukin-1 release. The inflammatory response occurred later than phagocytosis and, because carbon black had no effect on cytokine release, its extent appeared to depend on the content of adsorbed organic compounds. Furthermore, treatment of the exhaust gas to decrease the adsorbed organic fraction reduced the DPM-induced increase in GM-CSF factor release. These results indicate that DPM can be phagocytosed by and induce a specific inflammatory response in airway epithelial cells.

5.1.1.1.5. ***Summary*****.** In the available exposure studies, considerable variability is reported in diesel exhaust detection threshold. The odor scales described in some of these studies have no general use at present because they are not objectively defined; however, the studies do clearly indicate substantial interindividual variability in the ability to detect odor and the level at which it becomes objectionable. Much of what is known about the acute effects of diesel exhaust comes from case reports that lack clear measurements of exposure concentrations. The studies of pulmonary function changes in exposed humans have looked for changes occurring over a workshift or after a short-term exposure. The overall conclusion of these studies is that reversible changes in pulmonary function in humans can occur in relation to diesel exhaust exposure,

although it is not possible to relate these changes to specific exposure levels. Exposure studies in humans and in isolated cell systems derived from humans reveal that DPM has the potential to elicit inflammatory and immunological responses and responses typical of asthma; DPM may be a likely factor in the increasing incidence of allergic hypersensitivity. These studies have also shown that effects are due primarily to the organic fraction and that DPM synergizes with known allergens to increase their effectiveness. Results from human cell culture studies indicate that DPM has the potential to influence the development of airway inflammation and disease through its adjuvant properties and by causing the release of proinflammatory mediators.

5.1.1.2. *Long-Term Exposures*

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers.

Battigelli et al. (1964) measured several indices of pulmonary function, including vital capacity, FEV_1, peak flow, nitrogen washout, and diffusion capacity in 210 locomotive repairmen exposed to diesel exhaust in 3 engine houses. The average exposure of these locomotive repairmen to diesel exhaust was 9.6 years. When compared with a control group matched for age, body size, "past extrapulmonary medical history" (no explanation given), and job status (154 railroad yard workers), no significant clinical differences were found in pulmonary function or in the prevalence of dyspnea, cough, or sputum between the diesel exhaust-exposed and nonexposed groups. Exposure to diesel exhaust showed marked seasonal variations because the doors of the engine house were open in the summer and closed in the winter. For the exposed group, the maximum daily workplace concentrations of air pollutants measured were 1.8 ppm NO_2, 1.7 ppm total aldehydes, 0.15 ppm acrolein, 4.0 ppm SO_2, and 5.0 ppm total hydrocarbons. The concentration of airborne particles was not reported.

Gamble et al. (1987b) examined 283 diesel bus garage workers from four garages in two cities to determine if there was excess chronic respiratory morbidity associated with exposure to diesel exhaust. Tenure of employment was used as a surrogate of exposure; mean tenure of the study population was 9 years ± 10 years S.D. Exposure-effect relationships within the study population showed no detectable associations of symptoms with tenure. Reductions in FVC, FEV_1, peak flow, and FEF_{50} (but not FEF_{75}) were associated with increasing tenure. Compared with a control population (716 nonexposed blue-collar workers) and after indirect adjustment for age, race, and smoking, the exposed workers had a higher incidence of cough, phlegm, and wheezing; however, there was no correlation between symptoms and length of employment. Dyspnea showed an exposure-response trend but no apparent increase in prevalence. Mean FEV_1, FVC, FEF_{50}, and peak flow were not reduced in the total cohort compared with the reference population, but were reduced in workers with 10 years or more tenure.

Purdham et al. (1987) evaluated respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations who were exposed to both diesel and gasoline exhausts and in a control group of 11 on-site office workers. Twenty-four percent of the exposed group and 36% of the controls were smokers. If a particular symptom was considered to be influenced by smoking, smoking status was used as a covariate in the logistic regression analysis; pack-years smoked was a covariate for lung function indices. The frequency of respiratory symptoms was not significantly different between the two groups; however, baseline pulmonary function measurements were significantly different. The latter comparisons were measured by multiple regression analysis using the actual (not percentage predicted) results and correcting for age, height, and pack-years smoked. The stevedores had significantly lower FEV_1, FEV_1/FVC, FEF_{50}, and FEF_{75} (p<0.021, p<0.023, p<0.001, and p<0.008, respectively), but not FVC. The results from the stevedores were also compared with those obtained from a study of the respiratory health status of Sydney, Nova Scotia, residents. These comparisons showed that the dock workers had higher FVC, similar FEV_1, but lower FEV_1/FVC and flow rates than the residents of Sydney. Based on these consistent findings, the authors concluded that the lower baseline function measurements in the stevedores provided evidence of an obstructive ventilatory defect, but caution in interpretation was warranted because of the small sample size. There were no significant changes in lung function over the workshift, nor was there a difference between the two groups. The stevedores were exposed to significantly (p<0.04) higher concentrations of particulate matter (0.06 to 1.72 mg/m^3, mean 0.50 mg/m^3) than the controls (0.13 to 0.58 mg/m^3, mean not reported). Exposures of stevedores to SO_2, NO_2, aldehydes, and PAHs were very low; occasional CO concentrations in the 20 to 100 ppm range could be detected for periods up to 1 h in areas where blockers were chaining gasoline-powered vehicles.

Additional epidemiological studies on the health hazards posed by exposure to diesel exhaust have been conducted for mining operations. Reger et al. (1982) evaluated the respiratory health status of 823 male coal miners from six diesel-equipped mines compared with 823 matched coal miners not exposed to diesel exhaust. The average tenure of underground work for the underground miners and their controls was only about 5 years; on average, the underground workers in diesel mines spent only 3 of those 5 years underground in diesel-use mines. Underground miners exposed to diesel exhaust reported a higher incidence of symptoms of cough and phlegm but proportionally fewer symptoms of moderate to severe dyspnea than their matched counterparts. These differences in prevalence of symptoms were not statistically significant. The diesel-exposed underground miners, on the average, had lower FVC, FEV_1, FEF_{50}, FEF_{75}, and FEF_{90} but higher peak flow and FEF_{25} than their matched controls. These differences, however, were not statistically significant. Health indicators for surface workers and their matched controls were directionally the same as for matched underground workers. There were no consistent

relationships between the findings of increased respiratory symptoms, decreased pulmonary function, smoking history, years of exposure, or monitored atmosphere pollutants (NO_x, CO, particles, and aldehydes). Mean concentrations of NO_x at the six mines ranged from 0 to 0.6 ppm for short-term area samples, 0.13 to 0.28 ppm for full-shift personal samples, and 0.03 to 0.80 for full-shift area samples. Inhalable particles (less than 10 μm MMAD) averaged 0.93 to 2.73 mg/m^3 for personal samples and 0 to 16.1 mg/m^3 for full-shift area samples. Ames et al. (1984), using a portion of the miners studied by Reger, examined 280 diesel-exposed underground miners in 1977 and again in 1982. Each miner in this group had at least 1 year of underground mining work history in 1977. The control group was 838 miners with no exposure to diesel exhaust. The miners were evaluated for prevalence of respiratory symptoms, chronic cough, phlegm, dyspnea, and changes in FVC, FEV_1, and FEF_{50}. No air monitoring data were reported; exposure to diesel exhaust gases and mine dust particles were described as very low. These authors found no decrements in pulmonary function or increased prevalence of respiratory symptoms attributable to exposure to diesel exhaust. In fact, the 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust.

Attfield (1978) studied 2,659 miners from 21 mines (8 metal, 6 potash, 5 salt, and 2 trona). Diesels were employed in only 18 of the mines, but the 3 mines not using diesels were not identified. The years of diesel usage, ranging from 8 in trona mines to 16 in potash mines, were used as a surrogate for exposure to diesel exhaust. Based on a questionnaire, an increased prevalence of persistent cough was associated with exposure to aldehydes; this finding, however, was not supported by the pulmonary function data. No adverse respiratory symptoms or pulmonary function impairments were related to CO_2, CO, NO_2, inhalable dust, or inhalable quartz. The author failed to comment on whether the prevalence of cough was related to the high incidence (70%) of smokers in the cohort.

Questionnaire, chest radiograph, and spirometric data were collected by Attfield et al. (1982) on 630 potash miners from six potash mines. These miners were exposed for an average of 10 years (range 5 to 14 years) to 0.1 to 3.3 ppm NO_2, 0.1 to 4.0 ppm aldehyde, 5 to 9 ppm CO, and total dust concentrations of 9 to 23 mg/m^3. No attempt was made to measure diesel-derived particles separately from other dusts. The ratio of total to inhalable (<10 μm MMAD) dust ranged from 2 to 11. An increased prevalence of respiratory symptoms was related solely to smoking. No association was found between symptoms and tenure of employment, dust exposure, NO_2, CO, or aldehydes. A higher prevalence of symptoms of cough and phlegm was found, but no differences in pulmonary function (FVC and FEV_1) were found in these diesel-exposed potash miners when compared with the predicted values derived from a logistics model based on blue-collar workers working in nondusty jobs.

Gamble et al. (1983) investigated respiratory morbidity in 259 miners from 5 salt mines in terms of increased respiratory symptoms, radiographic findings, and reduced pulmonary function associated with exposure to NO_2, inhalable particles (<10 μm MMAD), or years worked underground. Two of the mines used diesel extensively; no diesels were used in one salt mine. Diesels were introduced into each mine in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt miner cohort. After adjustment for age and smoking, the salt miners showed no increased prevalence of cough, phlegm, dyspnea, or airway obstruction (FEV_1/FVC) compared with aboveground coal miners, potash miners, or blue-collar workers. The underground coal miners consistently had an elevated level of symptoms. Forced expiratory volume at 1 s, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in relation to all the comparison populations. There was, however, no association between changes in pulmonary function and years worked, estimated cumulative inhalable particles, or estimated NO_2 exposure. The highest average exposure to particulate matter was 1.4 mg/m^3 (particle size not reported, measurement includes NaCl). Mean NO_2 exposure was 1.3 ppm, with a range of 0.17 ppm to 2.5 ppm. In a continuation of these studies, Gamble and Jones (1983) grouped the salt miners into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exhaust exposure. Average concentrations of inhalable particles and NO_2 were 0.40, 0.60, and 0.82 mg/m^3 and 0.64, 1.77, and 2.21 ppm for the three diesel exposure categories, respectively. A statistically significant concentration-response association was found between the prevalence of phlegm in the salt miners and exposure to diesel exhaust (p<0.0001) and a similar, but nonsignificant, trend for cough and dyspnea. Changes in pulmonary function showed no association with diesel tenure. In a comparison with the control group of nonexposed, blue-collar workers, adjusted for age and smoking, the overall prevalence of cough and phlegm (but not dyspnea) was elevated in the diesel-exposed workers. Forced expiratory volumes at 1 s and FVC were within 4% of expected, which was considered to be within the normal range of variation for a nonexposed population.

In a preliminary study of three subcohorts from bus company personnel (clerks [lowest exposure], bus drivers [intermediate exposure], and bus garage workers [highest exposure]) representing different levels of exposure to diesel exhaust, Edling and Axelson (1984) found a fourfold higher risk ratio for cardiovascular mortality in bus garage workers, even after adjusting for smoking history and allowing for at least 10 years of exposure and 15 years or more of induction latency. Carbon monoxide was hypothesized as the etiologic agent for the increased cardiovascular disease but was not measured. However, in a more comprehensive epidemiological study, Edling et al. (1987) evaluated mortality data covering a 32-year period for a cohort of 694 bus garage employees and found no significant differences between the observed

and expected number of deaths from cardiovascular disease. Information on exposure components and their concentrations was not reported.

The absence of reported noncancerous human health effects, other than infrequently occurring effects related to respiratory symptoms and pulmonary function changes, is notable. Unlike studies in laboratory animals, to be described later in this chapter, studies of the impact of diesel exhaust on the defense mechanisms of the human lung have not been performed. No direct evidence is available in humans regarding doses of diesel exhaust, gas phase, particulate phase, or total exhaust that lead to impaired particle clearance or enhanced susceptibility to infection. A summary of epidemiology studies is presented in Table 5-1.

To date, no large-scale epidemiological study has looked for effects of chronic exposure to diesel exhaust on pulmonary function. In the long-term longitudinal and cross-sectional studies, a relationship was generally observed between work in a job with diesel exposure and respiratory symptoms (such as cough and phlegm), but there was no consistent effect on pulmonary function. The interpretation of these results is hampered by lack of measured diesel exhaust exposure levels and the short duration of exposure in these cohorts. The studies are further limited in that only active workers were included, and it is possible that workers who have developed symptoms or severe respiratory disease are likely to have moved away from these jobs. The relationship between work in a job with diesel exposure and respiratory symptoms may be due to short-term exposure.

5.1.2. Laboratory Animal Studies

Because humans and laboratory animals show similar nonneoplastic responses to inhaled particles (ILSI, 2000), animal studies have been conducted to assess the pathophysiologic effects of DPM. Because of the large number of statistical comparisons made in the laboratory animal studies, and to permit uniform, objective evaluations within and among studies, data will be reported as significantly different (i.e., $p<0.05$) unless otherwise specified. The exposure regimens used and the resultant exposure conditions employed in the laboratory animal inhalation studies are summarized in Tables 5-2 through 5-16. Other than the pulmonary function studies performed by Wiester et al. (1980) on guinea pigs during their exposure in inhalation chambers, the pulmonary function studies performed by other investigators, although sometimes unreported, were interpreted as being conducted on the following day or thereafter and not immediately following exposure.

5.1.2.1. ***Acute Exposures***

The acute toxicity of undiluted diesel exhaust to rabbits, guinea pigs, and mice was assessed by Pattle et al. (1957). Four engine operating conditions were used, and 4 rabbits, 10 guinea pigs, and 40 mice were tested under each exposure condition for 5 h (no controls were used). Mortality was assessed up to 7 days after exposure. With the engine operating under light load, the exhaust was highly irritating but not lethal to the test species, and only mild tracheal and lung damage was observed in the exposed animals. The exhaust contained 74 mg/m^3 DPM (particle size not reported), 560 ppm CO, 23 ppm NO_2, and 16 ppm aldehydes. Exhaust containing 5 mg/m^3 DPM, 380 ppm CO, 43 ppm NO_2, and 6.4 ppm aldehydes resulted in low mortality rates (mostly below 10%) and moderate lung damage. Exhaust containing 122 mg/m^3 DPM, 418 ppm CO, 51 ppm NO_2, and 6.0 ppm aldehydes produced high mortality rates (mostly above 50%) and severe lung damage. Exhaust containing 1,070 mg/m^3 DPM, 1,700 ppm CO, 12 ppm NO_2, and 154 ppm aldehydes resulted in 100% mortality in all three species. High CO levels, which resulted in a carboxyhemoglobin value of 60% in mice and 50% in rabbits and guinea pigs, were considered to be the main cause of death in the latter case. High NO_2 levels were considered to be the main cause of lung damage and mortality seen in the other three tests. Aldehydes and NO_2 were considered to be the main irritants in the light load test.

Kobayashi and Ito (1995) administered 1, 10, or 20 mg/kg DPM in phosphate-buffered saline to the nasal mucosa of guinea pigs. The administration increased nasal airway resistance, augmented increased airway resistance and nasal secretion induced by a histamine aerosol, increased vascular permeability in dorsal skin, and augmented vascular permeability induced by histamine. The increases in nasal airway resistance and secretion are considered typical responses of nasal mucosa against allergic stimulation. Similar results were reported for guinea pigs exposed via inhalation for 3 h to diesel exhaust diluted to DPM concentrations of either 1 or 3.2 mg/m^3 (Kobayashi et al., 1997). These studies show that short-term exposure to DPM augments nasal mucosal hyperresponsiveness induced by histamine in guinea pigs.

The effects of DPM and its components (extracted particles and particle extracts) on the release of proinflammatory cytokines, interleukin-1 (IL-1), and tumor necrosis factor- (TNF-) by alveolar macrophages (AMs) were investigated by Yang et al. (1997). Rat AMs were incubated with 0, 5, 10, 20, 50, or 100 μg/10^6 AM/mL of DPM, methanol-extracted DPM, or equivalent concentrations of DPM at 37 ºC for 24 h. At high concentrations, both DPM and DPM extracts were shown to increase IL-1-like activity secreted by AMs, whereas extracted particles had no effect. Neither particles, particle extracts, or extracted particles stimulated secretion of TNF- . DPM inhibited lipid polysaccharide (LPS)-stimulated production of IL-1 and TNF- . In contrast, interferon (IFN)- stimulated production of TNF- was not affected by DPM. Results of this study indicate that the organic fraction of exhaust particles is responsible

for the effects noted. Stimulation of IL-1 but not TNF- suggests that IL-1, but not TNF- , may play an important role in the development of DPM-induced inflammatory and immune responses. The cellular mechanism involved in inhibiting increased release of IL-1 and TNF- by LPS is unknown, but may be a contributing factor to the decreased AM phagocytic activity and increased susceptibility to pulmonary infection after prolonged exposure to DPM.

Takano et al. (1997) designed a study to evaluate the effects of DPM on the manifestations of allergic asthma in mice, with emphasis on antigen-induced airway inflammation, the local expression of IL-5, GM-CSF, IL-2 and IFN- , and the production of antigen-specific IgE and IgG. Male ICR mice were intratracheally instilled with ovalbumin (OVA), DPM, and DPM+OVA. DPM was obtained from a 4JB1-type, light-duty 2.74 L, four-cylinder Izuzu diesel engine operated at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). The OVA-group mice were instilled with 1 μg OVA at 3 and 6 weeks. The mice receiving DPM alone were instilled with 100 μg DPM weekly for 6 weeks. The OVA + DPM group received the combined treatment in the same protocol as the OVA and the DPM groups, respectively. Additional groups were exposed for 9 weeks. DPM aggravated OVA-induced airway inflammation, characterized by infiltration of eosinophils and lymphocytes and an increase in goblet cells in the bronchial epithelium. DPM in combination with antigen markedly increased IL-5 protein levels in lung tissue and bronchoalveolar lavage supernatants compared with either antigen or DPM alone. The combination of DPM and antigen induced significant increases in local expression of IL-4, GM-CSF, and IL-2, whereas expression of IFN- was not affected. In addition, DPM exhibited adjuvant activity for the antigen-specific production of IgG and IgE.

5.1.2.2. *Short-Term and Subchronic Exposures*

A number of inhalation studies have employed a regimen of 20 h/day, 7 days/week for varying exposure periods up to 20 weeks to differing concentrations of airborne particulate matter, vapor, and gas concentrations of diluted diesel exhaust. Exposure regimens and characterization of gas-phase components for these studies are summarized in Table 5-2. Pepelko et al. (1980a) evaluated the pulmonary function of cats exposed under these conditions for 28 days to 6.4 mg/m^3 DPM. The only significant functional change observed was a decrease in maximum expiratory flow rate at 10% vital capacity. The excised lungs of the exposed cats appeared charcoal gray, with focal black spots visible on the pleural surface. Pathologic changes included a predominantly peribronchial localization of black-pigmented macrophages within the alveoli characteristic of focal pneumonitis or alveolitis.

The effects of a short-term diesel exhaust exposure on arterial blood gases, pH, blood buffering, body weight changes, lung volumes, and deflation pressure-volume (PV) curves of young adult rats were evaluated by Pepelko (1982a). Exposures were 20 h/day, 7 days/week for

8 days to a concentration of 6.4 mg/m^3 DPM in the nonirradiated exhaust (RE) and 6.75 mg/m^3 in the irradiated exhaust (IE). In spite of the irradiation, levels of gaseous compounds were not substantially different between the two groups (Table 5-2). Body weight gains were significantly reduced in the RE-exposed rats and to an even greater degree in rats exposed to IE. Arterial blood gases and standard bicarbonate were unaffected, but arterial blood pH was significantly reduced in rats exposed to IE. Residual volume and wet lung weight were not affected by either exposure, but vital capacity and total lung capacity were increased significantly following exposure to RE. The shape of the deflation PV curves were nearly identical for the control, RE, and IE groups.

In related studies, Wiester et al. (1980) evaluated pulmonary function in 4-day-old guinea pigs exposed for 20 h/day, 7 days/week for 28 days to IE having a concentration of 6.3 mg/m^3 DPM. When housed in the exposure chamber, pulmonary flow resistance increased 35%, and a small but significant sinus bradycardia occurred as compared with controls housed and measured in control air chambers (p<0.002). Respiratory rate, tidal volume, minute volume, and dynamic compliance were unaffected, as were lead-1 electrocardiograms.

A separate group of adult guinea pigs was necropsied after 56 days of exposure to IE, to diluted RE, or to clean air (Wiester et al., 1980). Exposure resulted in a significant increase in the ratio of lung weight to body weight (0.68% for controls, 0.78% for IE, and 0.82% for RE). Heart/body weight ratios were not affected by exposure. Microscopically, there was a marked accumulation of black pigment-laden AMs throughout the lung, with a slight to moderate accumulation in bronchial and carinal lymph nodes. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

White and Garg (1981) studied pathologic alterations in the lungs of rats (16 exposed and 8 controls) after exposure to diesel exhaust containing 6 mg/m^3 DPM. Two rats from the exposed group and one rat from the control group (filtered room air) were sacrificed after each exposure interval of 6 h and 1, 3, 7, 14, 28, 42, and 63 days; daily exposures were for 20 h and were 5.5 days/week. Evidence of AM recruitment and phagocytosis of diesel particles was found at the 6-h sacrifice; after 24 h of exposure there was a focal, scattered increase in the number of Type II cells. After 4 weeks of exposure, there were morphologic changes in size, content, and shape of AM, septal thickening adjacent to clusters of AMs, and an appearance of inflammatory cells, primarily within the septa. At 9 weeks of exposure, focal aggregations of particle-laden macrophages developed near the terminal bronchi, along with an influx of PMNs, Type II cell proliferation, and thickening of alveolar walls. The affected alveoli occurred in clusters that, for the most part, were located near the terminal bronchioles, but occasionally were focally located in

the lung parenchyma. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

Mauderly et al. (1981) exposed rats and mice by inhalation to diluted diesel exhaust for 545 h over a 19-week period on a regimen of 7 h/day, 5 days/week at concentrations of 0, 0.21, 1.02, or 4.38 mg/m^3 DPM. Indices of health effects were minimal following 19 weeks of exposure. There were no significant exposure-related differences in mortality or body weights of the rats or mice. There also were no significant differences in respiratory function (breathing patterns, dynamic lung mechanics, lung volumes, quasi-static PV relationships, forced expirograms, and CO-diffusing capacity) in rats; pulmonary function was not measured in mice. No effect on tracheal mucociliary or deep lung clearances were observed in the exposed groups. Rats, but not mice, had elevated immune responses in lung-associated lymph nodes at the two higher exposure levels. Inflammation in the lungs of rats exposed to 4.38 mg/m^3 DPM was indicated by increases in PMNs and lung tissue proteases. Histopathologic findings included AMs that contained DPM, an increase in Type II cells, and the presence of particles in the interstitium and tracheobronchial lymph nodes.

Kaplan et al. (1982) evaluated the effects of subchronic exposure to diesel exhaust on rats, hamsters, and mice. The exhaust was diluted to a concentration of 1.5 mg/m^3 DPM; exposures were 20 h/day, 7 days/week. Hamsters were exposed for 86 days, rats and mice for 90 days. There were no significant differences in mortality or growth rates between exposed and control animals. Lung weight relative to body weight of rats exposed for 90 days was significantly higher than the mean for the control group. Histological examination of tissues of all three species indicated particle accumulation in the lungs and mediastinal lymph nodes. Associated with the larger accumulations, there was a minimal increase in the thickness of the alveolar walls, but the vast majority of the particles elicited no response. After 6 mo of recovery, considerable clearance of the DPM from the lungs occurred in all three species, as evaluated by gross pathology and histopathology. However, no quantitative estimate of clearance was provided.

Toxic effects in animals from acute exposure to diesel exhaust appear to be primarily attributable to the gaseous components (i.e., mortality from CO intoxication and lung injury caused by cellular damage resulting from NO_2 exposure). The results from short-term exposures indicate that rats experience minimal lung function impairment even at diesel exhaust levels sufficiently high to cause histological and cytological changes in the lung. In subchronic studies of durations of 4 weeks or more, frank adverse health effects are not readily apparent and, when found, are mild and result from exposure to concentrations of about 6 mg/m^3 DPM and durations of exposures of 20 h/day. There is ample evidence that subchronic exposure to lower levels of

diesel exhaust affects the lung, as indicated by accumulation of particles, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and thickening of alveolar walls adjacent to AM aggregates. Little evidence exists, however, that subchronic exposure to diesel exhaust impairs lung function. Recent studies have implicated the organic fraction of DPM in the induction of respiratory allergic disease.

5.1.2.3. ***Chronic Exposures***

5.1.2.3.1. ***Effects on growth and longevity.*** Changes in growth, body weight, absolute or relative organ weights, and longevity can be measurable indicators of chronic toxic effects. Such effects have been observed in some, but not all, of the long-term studies conducted on laboratory animals exposed to diesel exhaust. There was limited evidence for an effect on survival in the published chronic animal studies; deaths occurred intermittently early in one study in female rats exposed to 3.7 mg/m^3 DPM; however, the death rate began to decrease after 15 mo, and the survival rate after 30 mo was slightly higher than that of the control group (Research Committee for HERP Studies, 1988). Studies of the effects of chronic exposure to diesel exhaust on survival and body weight or growth are detailed in Table 5-3.

Increased lung weights and lung-to-body weight ratios have been reported in rats, mice, and hamsters. These data are summarized in Table 5-4. In rats exposed for up to 36 weeks to 0.25 or 1.5 mg/m^3 DPM, lung wet weights (normalized to body weight) were significantly higher in the 1.5 mg/m^3 exposure group than control values after 12 weeks of exposure (Misiorowski et al., 1980). Rats and Syrian hamsters were exposed for 2 years (five 16-h periods per week) to diesel exhaust diluted to achieve concentrations of 0.7, 2.2, and 6.6 mg/m^3 DPM (Brightwell et al., 1986). At necropsy, a significant increase in lung weight was seen in both rats and hamsters exposed to diesel exhaust compared with controls. This finding was more pronounced in the rats in which the increase was progressive with both duration of exposure and particulate matter level. The increase was greatest at 30 mo (after the end of a 6-mo observation period in the high-concentration male group where the lung weight was 2.7 times the control and at 24 mo in the high-concentration female group [3.9 times control]). Heinrich et al. (1986a,b; see also Stöber, 1986) found a significant increase in wet and dry weights of the lungs of rats and mice exposed at 4.24 mg/m^3 DPM for 1 year in comparison with controls. After 2 years, the difference was a factor of 2 (mice) or 3 (rats). After the same exposure periods, the hamsters showed increases of 50% to 75%, respectively. Exposure to equivalent filtered diesel exhaust caused no significant effects in any of the species. Vinegar et al. (1980, 1981a,b) exposed hamsters to two levels of diesel exhaust with resultant concentrations of about 6 and 12 mg/m^3 DPM for 8 h/day, 7 days/week for 6 mo. Both exposures significantly increased lung weight and lung-weight to

body-weight ratios. The difference between lung weights of exposed and control hamsters exposed to 12 mg/m^3 DPM was approximately twice that of those exposed to 6 mg/m^3.

Heinrich et al. (1995) reported that rats exposed to 2.5 and 7 mg/m^3 DPM for 18 h/day, 5 days/week for 24 mo showed significantly lower body weights than controls starting at day 200 in the high-concentration group and at day 440 in the low-concentration group. Body weight in the low-concentration group was unaffected, as was mortality in any group. Lung weight was increased in the 7 mg/m^3 group starting at 3 mo and persisting throughout the study, while the 2.5 mg/m^3 group showed increased lung weight only at 22 and 24 mo of exposure. Mice (NMRI strain) exposed to 7 mg/m^3 in this study for 13.5 mo had no increase in mortality and insignificant decreases in body weight. Lung weights were dramatically affected, with increases progressing throughout the study from 1.5-fold at 3 mo to 3-fold at 12 mo. Mice (NMRI and C57BL/6N strains) were also exposed to 4.5 mg/m^3 for 23 mo. In NMRI mice, the body weights were reported to be significantly lower than controls, but the magnitude of the change is not reported, so biological significance cannot be assessed. Mortality was slightly increased, but statistical significance is not reported. The C57BL/6N mice showed minimal effects on body weight and mortality, which were not statistically significant. Lung weights were dramatically affected in both strains.

Nikula et al. (1995) exposed male and female F344 rats to DPM concentrations of 2.4 and 6.3 mg/m^3 for 16 h/day, 5 days/week for 23 mo in a study designed to compare the effects of DPM with those of carbon black. Significantly reduced survival was observed in males exposed to 6.3 mg/m^3 but not in females or at the lower concentration. Body weights were decreased by exposure to 6.3 mg/m^3 DPM in both male and female rats throughout the exposure period. Significant increases in lung weight were first seen at 6 mo in the high-exposure group and at 12 to 18 mo in the low-exposure group.

No evidence was found in the published literature that chronic exposure to diesel exhaust affected the weight of body organs other than the lung and heart (e.g., liver, kidney, spleen, or testes) (Table 5-4). Morphometric analysis of hearts from rats and guinea pigs exposed to 0.25, 0.75, or 1.5 mg/m^3 DPM 20 h/day, 5.5 days/week for 78 weeks revealed no significant alteration in mass at any exposure level or duration of exposure (Penney et al., 1981). The analysis included relative wet weights of the right ventricle, left ventricle, combined atria, and ratio of right to left ventricle. Vallyathan et al. (1986) found no significant differences in heart weights and the ratio of heart weight to body weight between rats exposed to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo and their respective clean-air chamber controls. No significant differences were found in the lungs, heart, liver, spleen, kidney, and testes of rats exposed for 52 weeks, 7 h/day, 5 days/week to diluted diesel exhaust containing 2 mg/m^3 DPM compared with their respective controls (Green et al., 1983).

5.1.2.3.2. ***Effects on pulmonary function***. The effect of long-term exposure to diesel exhaust on pulmonary function has been evaluated in laboratory studies of rats, hamsters, cats, and monkeys. These studies are summarized in Table 5-5, along with more details on the exposure characteristics, in general order of increasing dose (C × T) of DPM. The text will be presented using the same approach.

Lewis et al. (1989) evaluated functional residual capacity and airway resistance and conductance in 10 control and 10 diesel-exposed rats (2 mg/m^3 DPM, 7 h/day, 5 days/week for 52 or 104 weeks). At the 104-week evaluation, the rats were also examined for maximum flow volume impairments. No evidence of impaired pulmonary function as a result of the exposure to diesel exhaust was found in rats. Lewis et al. (1989) exposed male cynomolgus monkeys to diesel exhaust for 7 h/day, 5 days/week for 24 mo. Groups of 15 monkeys were exposed to air, diesel exhaust (2 mg/m^3), coal dust, or combined coal dust and diesel exhaust. Pulmonary function was evaluated prior to exposure and at 6-mo intervals during the 2-year exposure, including compliance and resistance, static and dynamic lung volumes, distribution of ventilation, diffusing capacity, and maximum ventilatory performance. There were no effects on lung volumes, diffusing capacity, or ventilation distribution, so there was no evidence of restrictive disease. There was, however, evidence of obstructive airway disease as measured by low maximal flows in diesel-exposed monkeys. At 18 mo of exposure, forced expiratory flow at 25% of vital capacity and forced expiratory flow normalized to FVC were decreased. The measurement of forced expiratory flow at 40% of total lung capacity was significantly decreased at 12, 18, and 24 mo of exposure. The finding of an obstructive effect in monkeys contrasts with the finding of restrictive type effects in other laboratory animal species (Vinegar et al., 1980, 1981a; Mauderly et al., 1988; Pepelko et al., 1980b, 1981) and suggests a possible difference in effect between primate and small animal respiratory tracts. In these monkeys there were no specific histopathological effects reported (see next section), although particle aggregates were reported in the distal airways, suggesting more small airway deposition.

Gross (1981) exposed rats for 20 h/day, 5.5 days/week for 87 weeks to diesel exhaust containing 1.5 mg/m^3 DPM. When the data were normalized (e.g., indices expressed in units of airflow or volume for each animal by its own forced expiratory volume), there were no apparent functionally significant changes occurring in the lungs at 38 weeks of exposure that might be attributable to the inhalation of diesel exhaust. After 87 weeks of exposure, functional residual capacity (FRC) and its component volumes (expiratory reserve [ER] and residual volume [RV]), maximum expiratory flow (MEF) at 40% FVC, MEF at 20% FVC, and $FEV_{0.1}$ were significantly greater in the diesel-exposed rats. An observed increase in airflow at the end of the forced expiratory maneuver when a decreased airflow would be expected from the increased FRC, ER, and RV data (the typical scenario of human pulmonary disease) showed these data to be

inconsistent with known clinically significant health effects. Furthermore, although the lung volume changes in the diesel-exposed rats could have been indicative of emphysema or chronic obstructive lung disease, this interpretation was contradicted by the airflow data, which suggest simultaneous lowering of the resistance of the distal airways.

Heinrich et al. (1982) evaluated the pulmonary function of rats exposed to a concentration of 3.9 mg/m^3 DPM for 7 to 8 h/day, 5 days/week for 2 years. When compared with a control group, no significant changes in respiratory rate, minute volume, compliance, or resistance occurred in the exposed group (number of rats per group was not stated).

Chinese hamsters (eight or nine per group) were exposed 8 h/day, 7 days/week, for 6 mo to concentrations of either about 6 mg/m^3 or about 12 mg/m^3 DPM (Vinegar et al., 1980, 1981a,b). Vital capacity, vital capacity/lung weight ratio, residual lung volume by water displacement, and CO_2 diffusing capacity decreased significantly in hamsters exposed to 6 mg/m^3 DPM. Static deflation volume-pressure curves showed depressed deflation volumes for diesel-exposed hamsters when volumes were corrected for body weight and even greater depressed volumes when volumes were corrected for lung weight. However, when volumes were expressed as percentage of vital capacity, the diesel-exposed hamsters had higher lung volumes at 0 and 5 cm H_2O. In the absence of confirmatory histopathology, the authors tentatively concluded that these elevated lung volumes and the significantly reduced diffusing capacity in the same hamsters were indicative of possible emphysematous changes in the lung. Similar lung function changes were reported in hamsters exposed at 12 mg/m^3 DPM, but detailed information was not reported. It was stated, however, that the decrease in vital capacity was 176% greater in the second experiment than in the first.

Mauderly et al. (1988; see also McClellan et al., 1986) examined the impairment of respiratory function in rats exposed for 7 h/day, 5 days/week for 24 mo to diluted diesel exhaust with 0.35, 3.5, or 7.1 mg/m^3 DPM. After 12 mo of exposure to the highest concentration of diesel exhaust, the exposed rats (n = 22) had lower total lung capacity (TLC), dynamic lung compliance (C_{dyn}), FVC, and CO diffusing capacity than controls (n = 23). After 24 mo of exposure to 7.1 mg/m^3 DPM, mean TLC, C_{dyn}, quasi-static chord compliance, and CO diffusing capacity were significantly lower than control values. Nitrogen washout and percentage of FVC expired in 0.1 s were significantly greater than control values. There was no evidence of airflow obstruction. The functional alterations were attributed to focal fibrotic and emphysematous lesions and thickened alveolar membranes observed by histological examination. Similar functional alterations and histopathologic lesions were observed in the rats exposed to 3.5 mg/m^3 DPM, but such changes usually occurred later in the exposure period and were generally less pronounced. There were no significant decrements in pulmonary function for the 0.35 mg/m^3 group at any time during the study nor were there reported histopathologic changes in this group.

Additional studies were conducted by Heinrich et al. (1986a,b; see also Stöber, 1986) on the effects of long-term exposure to diesel exhaust on the pulmonary function of hamsters and rats. The exhaust was diluted to achieve a concentration of 4.24 mg/m^3 DPM; exposures were for 19 h/day, 5 days/week for a maximum of 120 weeks (hamsters) or 140 weeks (rats). After 1 year of exposure to the diesel exhaust, the hamsters exhibited a significant increase in airway resistance and a nonsignificant reduction in lung compliance. For the same time period, rats showed increased lung weights, a significant decrease in C_{dyn}, and a significant increase in airway resistance. These indices did not change during the second year of exposure.

Syrian hamsters and rats were exposed to 0.7, 2.2, or 6.6 mg/m^3 DPM for five 16-h periods per week for 2 years (Brightwell et al., 1986). There were no treatment-related changes in pulmonary function in the hamster. Rats exposed to the highest concentration of diesel exhaust exhibited changes in pulmonary function (data not presented) that were reported to be consistent with a concentration-related obstructive and restrictive disease.

Pepelko et al. (1980b; 1981; see also Pepelko, 1982b) and Moorman et al. (1985) measured the lung function of adult cats chronically exposed to diesel exhaust. The cats were exposed for 8 h/day and 7 days/week for 124 weeks. Exposures were at 6 mg/m^3 for the first 61 weeks and 12 mg/m^3 from weeks 62 to 124. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes (TLC, FVC, FRC, and inspiratory capacity [IC]) and the significantly lower single-breath diffusing capacity, coupled with normal values for dynamic ventilatory function (mechanics of breathing), indicate the presence of a lesion that restricts inspiration but does not cause airway obstruction or loss of elasticity. This pulmonary physiological syndrome is consistent with an interstitial fibrotic response that was later verified by histopathology (Plopper et al., 1983).

Pulmonary function impairment has been reported in rats, hamsters, cats, and monkeys chronically exposed to diesel exhaust. In all species but the monkey, the pulmonary function testing results have been consistent with restrictive lung disease. The monkeys demonstrated evidence of small airway obstructive responses. The disparity between the findings in monkeys and those in rats, hamsters, and cats could be in part the result of increased particle retention in the smaller species resulting from (1) exposure to diesel exhaust that has higher airborne concentrations of gases, vapors, and particles and/or (2) longer duration of exposure. The nature of the pulmonary impairment is also dependent on the site of deposition and routes of clearance, which are determined by the anatomy and physiology of the test laboratory species and the exposure regimen. The data on pulmonary function effects raise the possibility that diesel exhaust produces small airway disease in primates compared with primarily alveolar effects in small

animals and that similar changes might be expected in humans and monkeys. Unfortunately, the available data in primates are too limited to draw clear conclusions.

5.1.2.3.3. ***Lung morphology, biochemistry, and lung lavage analysis***. Several studies have examined the morphological, histological, and histochemical changes occurring in the lungs of laboratory animals chronically exposed to diesel exhaust. The histopathological effects of diesel exposure in the lungs of laboratory animals are summarized in Table 5-6, ranked in order of C × T. Table 5-6 also contains an expanded description of exposures.

Kaplan et al. (1982) performed macroscopic and microscopic examinations of the lungs of rats, mice, and hamsters exposed for 20 h/day, 7 days/week for 3 mo to diesel exhaust containing 1.5 mg/m^3 DPM. Gross examination revealed diffuse and focal deposition of the diesel particles that produced a grayish overall appearance of the lungs with scattered, denser black areas. There was clearance of particles via the lymphatics to regional lymph nodes. Microscopic examination revealed no anatomic changes in the upper respiratory tract; the mucociliary border was normal in appearance. Most of the particles were in macrophages, but some were free as small aggregates on alveolar and bronchiolar surfaces. The particle-laden macrophages were often in masses near the entrances of the lymphatic drainage and respiratory ducts. Associated with these masses was a minimal increase in the thickness of the alveolar walls; however, the vast majority of the particles elicited no response. After 6 mo of recovery, the lungs of all three species contained considerably less pigment, as assessed by gross pathological and histopathological examinations.

Lewis et al. (1989; see also Green et al., 1983) performed serial histological examinations of rat lung tissue exposed to diesel exhaust containing 2 mg/m^3 DPM for 7 h/day, 7 days/week for 2 years. Accumulations of black-pigmented AMs were seen in the alveolar ducts adjacent to terminal bronchioles as early as 3 mo of exposure, and particles were seen within the interstitium of the alveolar ducts. These macular lesions increased in size up to 12 mo of exposure. Collagen or reticulum fibers were seen only rarely in association with deposited particles; the vast majority of lesions showed no evidence of fibrosis. There was no evidence of focal emphysema with the macules. Multifocal histiocytosis (24% of exposed rats) was observed only after 24 mo of exposure. These lesions were most commonly observed subpleurally and were composed of collections of degenerating macrophages and amorphous granular material within alveoli, together with fibrosis and chronic inflammatory cells in the interstitium. Epithelial lining cells adjacent to collections of pigmented macrophages showed a marked Type II cell hyperplasia; degenerative changes were not observed in Type I cells. Histological examination of lung tissue from monkeys exposed for 24 mo in the same regimen as used for rats revealed aggregates of black particles, principally in the distal airways of the lung. Particles were present within the cytoplasm of macrophages in the alveolar spaces as well as the interstitium. Fibrosis, focal emphysema, or

inflammation was not observed. No specific histopathological lesions were reported for the monkey.

Nikula et al. (1997) reevaluated the lung tissue from this study. They concluded that there were no significant differences in the amount of retained particulate matter between monkeys and rats exposed under the same conditions. The rats, however, retained a greater portion of the particulate matter in lumens of the alveolar ducts and alveoli than did the monkeys. Conversely, monkeys retained a greater portion of the particulate material in the interstitium than did rats. Aggregations of particle-laden macrophages in the alveoli were rare, and there were few signs of particle-associated inflammation in the monkeys. Minimal histopathologic lesions were detected in the interstitium.

Histopathological effects of diesel exhaust on the lungs of rats have been investigated by the Health Effects Research Program on Diesel Exhaust (HERP) in Japan. Both light-duty (LD) and heavy-duty (HD) diesel engines were used. The exhaust was diluted to achieve nominal concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD only) mg/m^3 DPM. Rats were exposed for 16 h/day, 6 days/week for 30 mo. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 DPM or less. At concentrations above 0.4 mg/m^3 DPM, severe morphological changes were observed. These changes consisted of shortened and absent cilia in the tracheal and bronchial epithelium, marked hyperplasia of the bronchiolar epithelium, and swelling of the Type II cellular epithelium. These lesions appeared to increase in severity with increases in exhaust concentration and duration of exposure. There was no difference in the degree of changes in pulmonary pathology at the same concentrations between the LD and the HD series.

Heinrich et al. (1982) investigated histological changes occurring in the respiratory tract of hamsters exposed to diesel exhaust. Exposures were for 7 to 8 h/day, 5 days/week for 104 weeks to diesel exhaust diluted to achieve a concentration of 3.9 mg/m^3 DPM. Significantly higher numbers of hamsters in the group exposed to diesel exhaust exhibited definite proliferative changes in the lungs compared with the groups exposed to particle-free diesel exhaust or clean air. Sixty percent of these changes were described as adenomatous proliferations.

Heinrich et al. (1995) reported increased incidence and severity of bronchioloalveolar hyperplasia in rats exposed to 0.8, 2.5, and 7 mg/m^3. The lesion in the lowest concentration group was described as very slight to moderate. Slight to moderate interstitial fibrosis also increased in incidence and severity in all exposed groups, but incidences were not reported. This chronic study also exposed NMRI mice to 7 mg/m^3 for 13.5 mo and both NMRI and C56BL/6N mice to 4.5 mg/m^3 for 24 mo. Noncancer histological endpoints are not discussed in any detail in the report, which is focused on the carcinogenicity of diesel as compared with titanium dioxide and carbon black.

Iwai et al. (1986) performed serial histopathology on the lungs of rats at 1, 3, 6, 12, and 24 mo of exposure to diesel exhaust. Exposures were for 8 h/day, 7 days/week for 24 mo; the exposure atmosphere contained 4.9 mg/m^3 DPM. At 1 and 3 mo of exposure, there were minimal histological changes in the lungs of the exposed rats. After 6 mo of exposure, there were particle-laden macrophages distributed irregularly throughout the lung and a proliferation of Type II cells with adenomatous metaplasia in areas where the macrophages had accumulated. After 1 year of exposure, foci of heterotrophic hyperplasia of ciliated or nonciliated bronchiolar epithelium on the adjacent alveolar walls were more common, the quantity of deposited particulate matter increased, and the number of degenerative AMs and proliferative lesions of Type II or bronchiolar epithelial cells increased. After 2 years of exposure, there was a fibrous thickening of the alveolar walls, mast cell infiltration with epithelial hyperplasia in areas where the macrophages had accumulated, and neoplasms.

Heinrich et al. (1986a; see also Stöber, 1986) performed histopathologic examinations of the respiratory tract of hamsters, mice, and rats exposed to diesel exhaust that had 4 mg/m^3 DPM. Exposures were for 19 h/day, 5 days/week; the maximum exposure period was 120 weeks for hamsters and mice and 140 weeks for rats. Histological examination revealed different levels of response among the three species. In hamsters, the exhaust produced thickened alveolar septa, bronchioloalveolar hyperplasia, and what were termed emphysematous lesions (diagnostic methodology not described). In mice, bronchoalveolar hyperplasia occurred in 64% of the mice exposed to the exhaust and in 5% of the controls. Multifocal alveolar lipoproteinosis occurred in 71% and multifocal interstitial fibrosis occurred in 43% of the mice exposed to exhaust but in only 4% of the controls. In exposed rats, there were severe inflammatory changes in the lungs, as well as thickened septa, foci of macrophages, and hyperplastic and metaplastic lesions.

Nikula et al. (1995) reported in detail the nonneoplastic effects in male and female F344 rats exposed to 2.4 or 6.3 mg/m^3 of DPM. At 3 mo in the low-concentration group, enlarged particle-containing macrophages were found with minimal aggregation. With higher concentration and longer duration of exposure, the number and size of macrophages and aggregates increased. Alveolar epithelial hyperplasia was found starting at 3 mo and in all rats at 6 mo. These lesions progressed to chronic active inflammation, alveolar proteinosis, and septal fibrosis at 12 mo. Other lesions observed late in the study included bronchiolar-alveolar metaplasia, squamous metaplasia, and squamous cysts. This study reports in detail the progression of lesions in diesel exhaust exposure and finds relatively little difference between the lesions caused by diesel exhaust exposure and exposure to similar levels of carbon black particles.

The effects of diesel exhaust on the lungs of rats exposed to 8.3 ± 2.0 mg/m^3 DPM were investigated by Karagianes et al. (1981). Exposures were for 6 h/day, 5 days/week, for 4, 8, 16, or 20 mo. Histological examinations of lung tissue noted focal aggregation of particle-laden

AMs, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema (diagnostic methodology not described). Lesion severity was related to length of exposure. No significant differences were noted in lesion severity among the diesel exhaust, the diesel exhaust plus coal dust (5.8 ± 3.5 mg/m^3), or the high-concentration (14.9 ± 6.2 mg/m^3) coal dust exposure groups following 20 mo of exposure.

Histological changes in the lungs of guinea pigs exposed to diluted diesel exhaust containing either 0.25, 0.75, 1.5, or 6.0 mg/m^3 DPM were reported by Barnhart et al. (1981; 1982). Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo resulted in an uptake of exhaust particles by three alveolar cell types (AMs, Type I cells, and interstitial macrophages) and also by granulocytic leukocytes (eosinophils). The alveolar-capillary membrane increased in thickness as a result of an increase in the absolute tissue volume of interstitium and Type II cells. In a continuation of these studies, guinea pigs were exposed to diesel exhaust (up to 6.0 mg/m^3 DPM) for 2 years (Barnhart et al., 1982). A minimal tissue response occurred at a concentration of 0.25 $mg/m^{3.}$ After 9 mo of exposure, there was a significant increase, about 30%, in Type I and II cells, endothelial cells, and interstitial cells over concurrent age-matched controls; by 24 mo only macrophages and Type II cells were significantly increased. As in the earlier study, ultrastructural evaluation showed that Type I cells, AMs, and eosinophils phagocytized the diesel particles. Exposure to 0.75 mg/m^3 for 6 mo resulted in fibrosis in regions of macrophage clusters and in focal Type II cell proliferation. No additional information was provided regarding the fibrotic changes with increasing concentration or duration of exposure. With increasing concentration/duration of diesel exhaust exposure, Type II cell clusters occurred in some alveoli. Intraalveolar debris was particularly prominent after exposures at 1.5 and 6.0 mg/m^3 and consisted of secretory products from Type II cells.

In studies conducted on hamsters, Pepelko (1982b) found that the lungs of hamsters exposed for 8 h/day, 7 days/week for 6 mo to 6 or 12 mg/m^3 DPM were characterized by large numbers of black AMs in the alveolar spaces, thickening of the alveolar epithelium, hyperplasia of Type II cells, and edema.

Lungs from rats and mice exposed to 0.35, 3.5, or 7.1 mg/m^3 (0.23 to 0.26 μm mass median diameter [MMD]) for 7 h/day and 5 days/week showed pathologic lesions (Mauderly et al., 1987a; Henderson et al., 1988). After 1 year of exposure at 7.1 mg/m^3, the lungs of the rats exhibited focal areas of fibrosis; fibrosis increased with increasing duration of exposure and was observable in the 3.5-mg/m^3 group of rats at 18 mo. The severity of inflammatory responses and fibrosis was directly related to the exposure level. In the 0.35 mg/m^3 group of rats, there was no inflammation or fibrosis. Although the mouse lungs contained higher burdens of diesel particles per gram of lung weight at each equivalent exposure concentration, there was substantially less inflammatory reaction and fibrosis than was the case in rats. Fibrosis was observed only in the

lungs of mice exposed at 7.1 mg/m^3 and consisted of fine fibrillar thickening of occasional alveolar septa.

Histological examinations were performed on the lungs of cats initially exposed to 6 mg/m^3 DPM for 61 weeks and subsequently increased to 12 mg/m^3 for Weeks 62 to 124 of exposure. Plopper et al. (1983; see also Hyde et al., 1985) concluded from the results of this study that exposure to diesel exhaust produced changes in both epithelial and interstitial tissue compartments and that the focus of these lesions in the peripheral lung was the centriacinar region where the alveolar ducts join the terminal conducting airways. This conclusion was based on the following evidence. The epithelium of the terminal and respiratory bronchioles in exposed cats consisted of three cell types (ciliated, basal, and Clara cells) compared with only one type (Clara cells) in the controls. The proximal acinar region showed evidence of peribronchial fibrosis and bronchiolar epithelial metaplasia. Type II cell hyperplasia was present in the proximal interalveolar septa. The more distal alveolar ducts and the majority of the rest of the parenchyma were unchanged from controls. Peribronchial fibrosis was greater at the end of 6 mo in clean air following exposure, whereas the bronchiolar epithelial metaplasia was most severe at the end of exposure. Following an additional 6 mo in clean air, the bronchiolar epithelium more closely resembled the control epithelial cell population.

Wallace et al. (1987) used transmission electron microscopy (TEM) to determine the effect of diesel exhaust on the intravascular and interstitial cellular populations of the lungs of exposed rats and guinea pigs. Exposed animals and matched controls were exposed to 0.25, 0.75, 1.5, or 6.0 mg/m^3 DPM for 2, 6, or 10 weeks or 18 mo. The results inferred the following: (1) exposure to 6.0 mg/m^3 for 2 weeks was insufficient to elicit any cellular response, (2) both species demonstrated an adaptive multicellular response to diesel exhaust, (3) increased numbers of fibroblasts were found in the interstitium from week 6 of exposure through month 18, and (4) there was no significant difference in either cell type or number in alveolar capillaries, but there was a significant increase at 18 mo in the mononuclear population in the interstitium of both species.

Additional means for assessing the adverse effects of diesel exhaust on the lung are to examine biochemical and cytological changes in bronchoalveolar lavage fluid (BALF) and in lung tissue. Fedan et al. (1985) performed studies to determine whether chronic exposure of rats affected the pharmacologic characteristics of rat airway smooth muscle. Concentration-response relationships for tension changes induced with acetylcholine, 5-hydroxytryptamine, potassium chloride, and isoproterenol were assessed in vitro on isolated preparations of airway smooth muscle (trachealis). Chronic exposure to diesel exhaust significantly increased the maximal contractile responses to acetylcholine compared with control values; exposure did not alter the

sensitivity (EC_{50} values) of the muscles to the agonists. Exposures were to diesel exhaust containing 2 mg/m^3 DPM for 7 h/day, 5 days/week for 2 years.

Biochemical studies of BALF obtained from hamsters and rats revealed that exposures to diesel exhaust caused significant increases in lactic dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), total protein, collagen, and protease (pH 5.1) after approximately 1 year and 2 years of exposure (Heinrich et al., 1986a). These responses were generally much greater in rats than in hamsters. Exposures were to diesel exhaust containing 4.24 mg/m^3 DPM for 19 h/day, 5 days/week for 120 (hamsters) to 140 (rats) weeks.

Protein, -glucuronidase activity, and acid phosphatase activity were significantly elevated in BALF obtained from rats exposed to diesel exhaust containing 0.75 or 1.5 mg/m^3 DPM for 12 mo (Strom, 1984). Exposure for 6 mo resulted in significant increases in acid phosphatase activity at 0.75 mg/m^3 and in protein, -glucuronidase, and acid phosphatase activity at the 1.5 mg/m^3 concentration. Exposure at 0.25 mg/m^3 DPM did not affect the three indices measured at either time period. The exposures were for 20 h/day, 5.5 days/week for 52 weeks.

Additional biochemical studies (Misiorowski et al., 1980) were conducted on laboratory animals exposed under the same conditions and at the same site as reported on by Strom (1984). In most cases, exposures at 0.25 mg/m^3 did not cause any significant changes. The DNA content in lung tissue and the rate of collagen synthesis were significantly increased at 1.5 mg/m^3 DPM after 6 mo. Collagen deposition was not affected. Total lung collagen content increased in proportion to the increase in lung weight. The activity of prolyl hydroxylase was significantly increased at 12 weeks at 0.25 and 1.5 mg/m^3; it then decreased with age. Lysal oxidase activity did not change. After 9 mo of exposure, there were significant increases in lung phospholipids in rats and guinea pigs exposed to 0.75 mg/m^3 and in lung cholesterol in rats and guinea pigs exposed to 1.5 mg/m^3. Pulmonary prostaglandin dehydrogenase activity was stimulated by an exposure at 0.25 mg/m^3 but was not affected by exposure at 1.5 mg/m^3 (Chaudhari et al., 1980, 1981). Exposures for 12 or 24 weeks resulted in a concentration-dependent lowering of this enzyme activity. Exposure of male rats and guinea pigs at 0.75 mg/m^3 for 12 weeks did not cause any changes in glutathione levels of the lung, heart, or liver. Rats exposed for 2 mo at 6 mg/m^3 showed a significant depletion of hepatic glutathione, whereas the lung showed an increase of glutathione (Chaudhari and Dutta, 1982). Schneider and Felt (1981) reported that similar exposures did not substantially change adenylate cyclase and guanylate cyclase activities in lung or liver tissue of exposed rats and guinea pigs.

Bhatnagar et al. (1980; see also Pepelko, 1982a) evaluated changes in the biochemistry of lung connective tissue of diesel-exposed rats and mice. The mice were exposed for 8 h/day and 7 days/week for up to 9 mo to exhaust containing 6 mg/m^3 DPM. Total lung protein content was measured, as was labeled proline and labeled leucine. Leucine incorporation is an index of total

protein synthesis, although collagen is very low in leucine. Proline incorporation reflects collagen synthesis. Amino acid incorporation was measured in vivo in the rat and in short-term organ culture in mice. Both rats and mice showed a large increase in total protein (41% to 47% in rats), while leucine incorporation declined and proline incorporation was unchanged. These data are consistent with an overall depression of protein synthesis in diesel-exposed animals and also with a relative increase in collagen synthesis compared to other proteins. The increase in collagen synthesis suggested proliferation of connective tissue and possible fibrosis (Pepelko, 1982a).

A number of reports (McClellan et al., 1986; Mauderly et al., 1987a, 1990a; Henderson et al., 1988) have addressed biochemical and cytological changes in lung tissue and BALF of rodents exposed for 7 h/day, 5 days/week for up to 30 mo at concentrations of 0, 0.35, 3.5, or 7.1 mg/m^3 DPM. At the lowest exposure level (0.35 mg/m^3), no biochemical or cytological changes occurred in the BALF or in lung tissue in either Fischer 344 rats or CD-1 mice. Henderson et al. (1988) provide considerable time-course information on inflammatory events taking place throughout a chronic exposure. A chronic inflammatory response was seen at the two higher exposure levels in both species, as evidenced by increases in inflammatory cells (macrophages and neutrophils), cytoplasmic and lysosomal enzymes (lactate dehydrogenase, glutathione reductase, and -glucuronidase), and protein (hydroxyproline) in BALF. Analysis of lung tissue indicated similar changes in enzyme levels as well as an increase in total lung collagen content. After 18 mo of exposure, lung tissue glutathione was depleted in a concentration-dependent fashion in rats but was slightly increased in mice. Lavage fluid levels of glutathione and glutathione reductase activity increased in a concentration-dependent manner and were higher in mice than in rats.

Rats exposed for up to 17 days to diluted diesel exhaust (3.5 mg/m^3 DPM) had a fivefold increase in the bronchoconstrictive prostaglandin $PGF_{2\alpha}$ and a twofold increase in the inflammatory leukotriene LTB_4. In similarly exposed mice, there was a twofold increase in both parameters. These investigators (Henderson et al., 1988) concluded that the release of larger amounts of such mediators of inflammation from the alveolar phagocytic cells of rats accounted for the greater fibrogenic response seen in that species.

Biochemical analysis of lung tissue from cats exposed for 124 weeks and held in clean air for an additional 26 weeks indicated increases of lung collagen; this finding was confirmed by an observed increase in total lung wet weight and in connective tissue fibers estimated morphometrically (Hyde et al., 1985). Exposures were for 7 h/day, 5 days/week at 6 mg/m^3 DPM for 61 weeks and at 12 mg/m^3 for weeks 62 to 124.

Heinrich et al. (1995) reported on bronchoalveolar lavage in animals exposed for 24 mo and found exposure-related increases in lactate dehydrogenase, -glucuronidase, protein, and

hydroxyproline in groups exposed to 2.5 or 7 mg/m^3, although detailed data are not presented. Lavage analyses were not carried out in concurrent studies in mice.

The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the interaction of diesel particles with airway epithelial cells and phagocytosis by AMs. The airway epithelial cells and activated macrophages release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of DPM increases, there is an aggregation of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type II cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes. The neutrophils and macrophages release mediators of inflammation and oxygen radicals that deplete a biochemical defense mechanism of the lung (i.e., glutathione). As will be described later in more detail, other defense mechanisms are affected, particularly the decreased viability of AMs, which leads to decreased phagocytic activity and death of the macrophage. The latter series of events may result in the presence of pulmonary inflammatory, fibrotic, or emphysematous lesions. The data suggest that there may be a threshold of exposure to diesel exhaust below which adverse structural and biochemical effects may not occur in the lung; however, differences in the anatomy and pathological responses of laboratory animals coupled with their lifespans compared with humans make a determination of human levels of exposure to diesel exhaust without resultant pulmonary injury a difficult and challenging endeavor.

5.1.2.3.4. ***Effects on pulmonary defense mechanisms.*** The respiratory system has a number of defense mechanisms that negate or compensate for the effects produced by the injurious substances that repeatedly insult the upper respiratory tract, the tracheobronchial airways, and the alveoli. The effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals as well as more details on exposure atmosphere are summarized in Table 5-7 and ranked by cumulative exposure (C × T).

Several studies have been conducted investigating the effect of inhaled diesel exhaust on the deposition and fate of inert tracer particles or diesel particles themselves. Lung clearance of deposited particles occurs in two distinct phases: a rapid phase (hours to days) from the tracheobronchial region via the mucociliary escalator and a much slower phase (weeks to months) from the nonciliated pulmonary region via, primarily but not solely, AMs. Battigelli et al. (1966) reported impaired tracheal mucociliary clearance in vitro in excised trachea from rats exposed for single or repeated exposures of 4 to 6 h at two dilutions of diesel exhaust that resulted in exposures of approximately 8 and 17 mg/m^3 DPM. The exposure to 17 mg/m^3 resulted in decreased clearance after a single exposure as well as after a cumulative exposure of 34 or 100 h. Clearance was reduced to a lesser extent and in fewer tracheas from animals exposed to 8 mg/m^3

for a cumulative exposure of 40 h. Lewis et al. (1989) found no difference in the clearance of $^{59}Fe_3O_4$ particles (1.5 μm MMAD, g 1.8) 1 day after dosing control and diesel exhaust-exposed rats (2 mg/m^3, 7 h/day, 5 days/week for 8 weeks).

Wolff et al. (1987) and Wolff and Gray (1980) studied the effects of both subchronic and chronic diesel exhaust exposure on the tracheal clearance of particles. Tracheal clearance assessments were made by measuring the retention of radiolabeled technetium macroaggregated-albumin remaining 1 h after instillation in the distal trachea of rats. In the subchronic studies, rats were exposed to 4.5, 1.0, or 0.2 mg/m^3 DPM on a 7 h/day, 5 days/week schedule for up to 12 weeks. After 1 week there was an apparent speeding of tracheal clearance at the 4.5 mg/m^3 exposure level (p=0.10), which returned toward baseline after 6 weeks and was slightly below the baseline rate at 12 weeks. In the 1.0 mg/m^3 group, there was a progressive significant reduction in the clearance rate at 6 and 12 weeks of exposure. There was a trend toward reduced clearance in the 0.2 mg/m^3 group. Scanning electron micrographs indicated minimal changes in ciliary morphology; however, there was an indication of a lower percentage of ciliated cells at the 1.0 and 4.5 mg/m^3 levels. In the chronic studies, rats were exposed to 0, 0.35, 3.5, or 7.1 mg/m^3 for 7 h/day, 5 days/week for 30 mo. There were no significant differences in tracheal clearance rates between the control group and any of the exposure groups after 6, 12, 18, 24, or 30 mo of exposure. The preexposure measurements for all groups, however, were significantly lower than those during the exposure period, suggesting a possible age effect. The preexposure value for the 3.5-mg/m^3 group was also significantly lower than the control group.

There is a substantial body of evidence for an impairment of particle clearance from the bronchiole-alveolar region of rats following exposure to diesel exhaust. Griffis et al. (1983) exposed rats 7 h/day, 5 days/week for 18 weeks to diesel exhaust at 0.15, 0.94, or 4.1 mg/m^3 DPM. Lung burdens of the 0.15, 0.94, and 4.1 mg/m^3 levels were 35, 220, and 1,890 μg/g lung, respectively, 1 day after the 18-week exposure. The clearance half-time of the DPM was significantly greater, almost double, for the 4.1 mg/m^3 exposure group than for those of the lower exposure groups, 165 ± 8 days versus 99 ± 8 days (0.94 mg/m^3) and 87 ± 28 days (0.15 mg/m^3), respectively.

Chan et al. (1981) showed a dose-related slowing of ^{14}C-diesel particle clearance in rats preexposed to diesel exhaust at 0.25 or 6 mg/m^3 particulate matter for 20 h/day, 7 days/week for 7 to 112 days. Clearance was inhibited in the 6 mg/m^3 group when compared by length of exposure or compared with the 0.25 mg/m^3 or control rats at the same time periods.

Heinrich et al. (1982) evaluated lung clearance in rats exposed for approximately 18 mo at 3.9 mg/m^3 DPM for 7 to 8 h/day, 5 days/week. Following exposure to $^{59}Fe_2O_3$-aerosol, the rats were returned to the diesel exhaust exposure and the radioactivity was measured over the thoracic

area at subsequent times. The biological half-life of the iron oxide deposited in the rats' lungs was nearly twice that of controls.

Heinrich also used labeled iron oxide aerosols to study clearance in rats exposed to 0.8, 2.5, or 7 mg/m^3 diesel DPM for 24 mo (Heinrich et al., 1995). Clearance measurements were carried out at 3, 12, and 18 mo of exposure. Half-times of clearance were increased in a concentration- and duration-related way in all exposed groups, with a range of a 50% increase in the 0.8 mg/m^3 group at 3 mo to an 11-fold increase in the 7 mg/m^3 group at 19 mo. The differential cell counts in these animals were stated to have shown clear effects in the 2.5 and 7 mg/m^3 groups, but specific information about the changes is not reported.

Wolff et al. (1987) investigated alterations in DPM clearance from the lungs of rats chronically exposed to diesel exhaust at 0, 0.35, 3.5, or 7.1 mg/m^3 DPM for 7 h/day, 5 days/week for up to 24 mo. Progressive increases in lung burdens were observed over time in all groups; levels of DPM in terms of milligrams per lung were 0.60, 11.5, and 20.5 after 24 mo of exposure at the 0.35, 3.5, or 7.1 mg/m^3 exposure levels, respectively. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of $^{67}Ga_2O_3$ (0.1 μm MMD) as early as 6 mo at the 7.1 mg/m^3 level and 18 mo at the 3.5 mg/m^3 level; no significant changes were seen at the 0.35 mg/m^3 level. Rats inhaled fused aluminosilicate particles (2 μm MMAD) labeled with ^{134}Cs after 24 mo of diesel exhaust exposure; long-term clearance half-times were 79, 81, 264, and 240 days for the 0, 0.35, 3.5, and 7.1 mg/m^3 groups, respectively. Differences were significant between the control and the 3.5 and 7.1 mg/m^3 groups ($p < 0.01$).

Mauderly et al. (1987b) compared the effects of diesel exhaust in the developing lung to the adult lung by exposing groups of male F344 rats to 3.5 mg/m^3 for 7 h/day, 5 days/week for 6 mo. One group (adult) was exposed between 6 and 12 mo of age, and the other was exposed beginning in utero and until 6 mo of age. Clearance of an inhaled monodisperse 2 μm aluminosilicate particle was measured after exposure for 6 mo. The clearance half-time of the slow phase was found to be doubled in adult rats compared with age-matched controls and was not significantly affected in developing rat lungs.

Mauderly et al. compared the effects of diesel exhaust in normal lungs with rats in which emphysema had been induced experimentally by instillation of elastase 6 weeks before diesel exhaust exposures. The rats were exposed to 3.5 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Measurements included histopathology, clearance, pulmonary function, lung lavage, and immune response. In the rats that were not pretreated with elastase, there was a significant reduction in the number of macrophages recovered by pulmonary lavage in contrast to the increases in macrophages reported by Strom (1984) and Henderson et al. (1988). The half-time of the slow phase of clearance of inhaled, 1 μm, monodisperse particles was doubled in the exposure animals without elastase pretreatment. The elastase pretreatment did not affect

clearance in unexposed animals but significantly reduced the effect of diesel. The clearance half-time was significantly less in elastase-pretreated, diesel-exposed animals than in diesel-exposed normal animals. Many other effects measured in this study were also less affected by diesel exposure in elastase-treated animals. Measurements of lung burden of DPM showed that elastase-pretreated animals accumulated less than half as much DPM mass as normal animals exposed at the same time, suggesting that the difference in effect could be explained by differences in dose to the lung.

Lewis et al. (1989) conducted lung burden and $^{59}Fe_3O_4$ tracer studies in rats exposed for 12 and 24 mo to 2 mg/m^3 DPM (7 h/day, 5 days/week). The slope of the Fe_3O_4 clearance curve was significantly steeper than that of the controls, indicating a more rapid alveolar clearance of the deposited $^{59}Fe_3O_4$. After 120 days from the inhalation of the tracer particle, 19% and 8% of the initially deposited $^{59}Fe_3O_4$ were present in the lungs of control and diesel exhaust-exposed rats, respectively. The lung burden of DPM, however, increased significantly between 12 and 24 mo of exposure (0.52 to 0.97% lung dry weight), indicating a later dose-dependent inhibition of clearance.

Alveolar macrophages, because of their phagocytic and digestive capabilities, are one of the prime defense mechanisms of the alveolar region of the lung against inhaled particles. Thus, characterization of the effects of diesel exhaust on various properties of AMs provides information on the integrity or compromise of a key pulmonary defense mechanism. The physiological viability of AMs from diesel-exposed rats was assessed after 2 years of exposure by Castranova et al. (1985). The 7 h/day, 5 days/week exposure at 2 mg/m^3 DPM had little effect on the following: viability, cell number, oxygen consumption, membrane integrity, lysosomal enzyme activity, or protein content of the AMs. A slight decrease in cell volume, a decrease in chemiluminescence indicative of a decreased secretion of reactive oxygen species, and a decrease in ruffling of the cell membrane were observed. These findings could be reflective of an overall reduction in phagocytic activity.

Exposure to diesel exhaust has been reported both to increase the number of recoverable AMs from the lung (Strom, 1984; Vostal et al., 1982; Henderson et al., 1988) or to produce no change in numbers (Chen et al., 1980; Castranova et al., 1985). Strom (1984) found that in rats exposed to 0.25 mg/m^3 DPM for 20 h/day, 5.5 days/week for 6 mo or 1 year, as well as in the controls, BAL cells consisted entirely of AMs, with no differences in the cell counts in the lavage fluid. At the higher concentrations, 0.75 or 1.5 mg DPM/m^3, the count of AM increased proportionally with the exposure concentration; the results were identical for AMs at both 6 and 11 or 12 mo of exposure. The increase in AM counts was much larger after exposure to 1.5 mg/m^3 DPM for 6 mo than after exposure to 0.75 mg/m^3 for 1 year, although the total mass (calculated as C × T) of deposited particulate burden was the same. These data suggested to the

authors that the number of lavaged AMs was proportional to the mass influx of particles rather than to the actual DPM burden in the lung. These results further implied that there may be a threshold for the rate of mass influx of DPM into the lungs of rats above which there was an increased recruitment of AMs. Henderson et al. (1988) reported similar findings of significant increases of AMs in rats and mice exposed to 7.1 mg/m^3 DPM for 18 and 24 mo, respectively, for 7 h/day, 5 days/week, but not at concentrations of 3.5 or 0.35 mg/m^3 for the same exposure durations. Chen et al. (1980), using an exposure regimen of 0.25 and 1.5 mg/m^3 DPM for 2 mo and 20 h/day and 5.5 days/week, found no significant changes in absolute numbers of AMs from guinea pig BALF, nor did Castranova et al. (1985) in rat BALF following exposure to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 2 years.

A similar inflammatory response was noted by Henderson et al. (1988) and Strom (1984), as evidenced by an increased number of PMNs present in BALF from rodents exposed to diesel exhaust. Henderson et al. (1988) found these changes in rats and mice exposed to 7.1 and 3.5 mg/m^3 DPM for 7 h/day, 5 days/week. Significant increases in BALF PMNs were observed in mice at 6 mo of exposure and thereafter at the 7.1 and 3.5 mg/m^3 exposure levels, but in rats only the 7.1 mg/m^3 exposure level showed an increase in BALF PMNs at 6 mo of exposure and thereafter. Significant increases in BALF PMNs occurred in rats at 12, 18, and 24 mo of exposure to 3.5 mg/m^3 DPM. Although increases in PMNs were usually greater in mice in terms of absolute numbers, the PMN response in terms of increase relative to controls was only about one-third that of rats. Strom (1984) reported that the increased numbers of PMNs in BALF were proportional to the inhaled concentrations and/or duration of exposure. The PMNs also appeared to be affiliated with clusters of aggregated AMs rather than to the diesel particles per se. Proliferation of Type II cells likewise occurred in response to the formed aggregates of AMs (White and Garg, 1981).

The integrity of pulmonary defense mechanisms can also be ascertained by assessing if exposure to diesel exhaust affects colonization and clearance of pathogens and alters the response of the challenged animals to respiratory tract infections. Campbell et al. (1980, 1981) exposed mice to diesel exhaust followed by infectious challenge with *Salmonella typhimurium*, *Streptococcus pyogenes*, or A/PR8-3 influenza virus and measured microbial-induced mortality. Exposures to diesel exhaust were to 6 mg/m^3 DPM for 8 h/day, 7 days/week for up to 321 days. Exposure to diesel exhaust resulted in enhanced susceptibility to the lethal effects of *S. pyogenes* infection at all exposure durations (2 h, 6 h; 8, 15, 16, 307, and 321 days). Tests with *S. typhimurium* were inconclusive because of high mortality rates in the controls. Mice exposed to diesel exhaust did not exhibit an enhanced mortality when challenged with the influenza virus. Hatch et al. (1985) found no changes in the susceptibility of mice to Group C *Streptococcus* sp. infection following intratracheal injection of 100 μg of DPM suspended in unbuffered saline.

Hahon et al. (1985) assessed virus-induced mortality, virus multiplication with concomitant IFN levels (lungs and sera), antibody response, and lung histopathology in mice exposed to diesel exhaust prior to infectious challenge with Ao/PR/8/34 influenza virus. Weanling mice were exposed to diesel exhaust containing 2 mg/m^3 DPM for 7 h/day, 5 days/week. In mice exposed for 1, 3, and 6 mo, mortality was similar between the exposed and control mice. In mice exposed for 3 and 6 mo, however, there were significant increases in the percentage of mice having lung consolidation, higher virus growth, depressed IFN levels, and a fourfold reduction in hemagglutinin antibody levels; these effects were not seen after the 1-mo exposure.

The effects of diesel exhaust on the pulmonary defense mechanisms are determined by three critical factors related to exposure: the concentrations of the pollutants, the exposure duration, and the exposure pattern. Higher doses of diesel exhaust as determined by an increase in one or more of these three variables have been reported to increase the numbers of AMs, PMNs, and Type II cells in the lung, whereas lower doses fail to produce such changes. The single most significant contributor to the impairment of the pulmonary defense mechanisms appears to be an excessive accumulation of DPM, particularly as particle-laden aggregates of AMs. Such an accumulation would result from an increase in deposition and/or a reduction in clearance. The deposition of particles does not appear to change significantly following exposure to equivalent diesel exhaust doses over time. Because of the significant nonlinearity in particle accumulation between low and high doses of diesel exhaust exposure, coupled with no evidence of increased particle deposition, an impairment in one or more of the mechanisms of pulmonary defense appears to be responsible for the DPM accumulation and subsequent pathological sequelae. The time of onset of pulmonary clearance impairment was dependent both on the magnitude and on the duration of exposures. For example, for rats exposed for 7 h/day, 5 days/week for 104 weeks, the concentration needed to induce pulmonary clearance impairment appears to lie between 0.35 and 2.0 mg/m^3 DPM.

5.1.2.3.5. ***Effects on the immune system—inhalation studies.*** The effects of diesel exhaust on the immune system of guinea pigs were investigated by Dziedzic (1981). Exposures were to 1.5 mg/m^3 DPM for 20 h/day, 5.5 days/week for up to 8 weeks. There was no effect of diesel exposure when compared with matched controls for the number of B and T lymphocytes and null cells isolated from the tracheobronchial lymph nodes, spleen, and blood. Cell viability as measured by trypan blue exclusion was comparable between the exposed and control groups. The results of this study and others on the effects of exposure to diesel exhaust on the immune system are summarized in Table 5-8.

Mentnech et al. (1984) examined the effect of diesel exhaust on the immune system of rats. Exposures were to 2 mg/m^3 DPM for 7 h/day, 5 days/week for up to 2 years. Rats exposed for 12 and 24 mo were tested for immunocompetency by determining antibody-producing cells in the spleen 4 days after immunization with sheep erythrocytes. The proliferative response of splenic T-lymphocytes to the mitogens concanavalin A and phytohemagglutinin was assessed in rats exposed for 24 mo. There were no significant differences between the exposed and control animals. Results obtained from these two assays indicate that neither humoral immunity (assessed by enumerating antibody-producing cells) nor cellular immunity (assessed by the lymphocyte blast transformation assay) were markedly affected by the exposures.

Bice et al. (1985) evaluated whether or not exposure to diesel exhaust would alter antibody immune responses induced after lung immunization of rats and mice. Exposures were to 0.35, 3.5, or 7.1 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Chamber controls and exposed animals were immunized by intratracheal instillation of SRBCs after 6, 12, 18, or 24 mo of exposure. No suppression in the immune response occurred in either species. After 12, 18, and 24 mo of exposure, the total number of anti-SRBC IgM antibody forming cells (AFCs) was elevated in rats, but not in mice, exposed to 3.5 or 7.1 mg/m^3 DPM; after 6 mo of exposure, only the 7.1 mg/m^3 level was found to have caused this response in rats. The number of AFCs per 10^6 lymphoid cells in lung-associated lymph nodes and the levels of specific IgM, IgG, or IgA in rat sera were not significantly altered. The investigators concluded that the increased cellularity and the presence of DPM in the lung-associated lymph nodes had only a minimal effect on the immune and antigen filtration function of these tissues.

The effects of inhaled diesel exhaust and DPM have been studied in a murine model of allergic asthma (Takano et al., 1998a,b). ICR mice were exposed for 12 h/day, 7 days/week for 40 weeks to diesel exhaust (0.3, 1.0, or 3.0 mg/m^3). The mice were sensitized with ovalbumin (OA) after 16 weeks exposure and subsequently challenged with aerosol allergen (1% OA in isotonic saline for 6 min) at 3-week intervals during the last 24 weeks of exposure. Exposure to diesel exhaust enhanced allergen-related eosinophil recruitment to the submucosal layers of the airways and to the bronchoalveolar space, and increased protein levels of GM-CSF and IL-5 in the lung in a dose-dependent manner. In the diesel exhaust-exposed mice, increases in eosinophil recruitment and local cytokine expression were accompanied by goblet cell proliferation in the bronchial epithelium and airway hyperresponsiveness to inhaled acetylcholine. In contrast, mice exposed to clean air or diesel exhaust without allergen provocation showed no eosinophil recruitment to the submucosal layers of the airways or to the bronchoalveolar space, and few goblet cells in the bronchial epithelium. The authors concluded that daily inhalation of diesel exhaust can enhance allergen-related respiratory diseases such as allergic asthma, and that this effect may be mediated by the enhanced local expression of IL-5 and GM-CSF. The effect of

DPM on a second characteristic of allergic asthma, airway hyperresponsiveness, was examined by Takano et al. (1998b). Laboratory mice were administered OA, DPM, or OA and DPM combined by intratracheal instillation for 6 wk. Respiratory resistance (Rrs) after acetylcholine challenge was measured 24 h after the final instillation. Rrs was significantly greater in the mice treated with OA and DPM than in the other treatments. The authors concluded that DPM can enhance airway responsiveness associated with allergen exposure.

In a series of inhalation studies following earlier instillation studies, Miyabara and co-workers investigated whether inhalation of diesel exhaust could enhance allergic reactions in laboratory mice. C3H/Hen mice were exposed to diesel exhaust (3 mg DPM/m^3) by inhalation for 5 weeks (Miyabara et al., 1998b) and, after 7 days of exposure, were sensitized to OA injected intraperitoneally. At the end of the diesel exhaust exposure, the mice were challenged with an OA aerosol for 15 min. Diesel exhaust caused an increase in the numbers of neutrophils and macrophages in bronchoalveolar lavage fluid independent of OA sensitization, whereas a significant increase in eosinophil numbers occurred only after diesel exhaust exposure was combined with antigen challenge. Even though OA alone caused an increase in eosinophil numbers in lung tissue, this response was enhanced by diesel exhaust. Diesel exhaust exposure combined with OA sensitization enhanced the number of goblet cells in lung tissue, respiratory resistance, production of OA-specific IgE and Ig1 in the serum, and overexpression of IL-5 in lung tissue. In a second study, C3H/Hen mice were sensitized with OA injected intraperitoneally and then exposed to diesel exhaust by inhalation for 12 h/day for 3 mo (Miyabara et al., 1998a). After 3 weeks of diesel exhaust exposure, and every 3 weeks thereafter, the mice were challenged with an OA aerosol. Exposure to diesel exhaust with antigen challenge induced airway hyperresponsiveness and airway inflammation, which was characterized by increased numbers of eosinophils and mast cells in lung tissue. The increase in inflammatory cells was accompanied by an increase in goblet cells in the bronchial epithelium. Airway hyperresponsiveness, but not eosinophilic infiltration or increased goblet cells, was increased by diesel exhaust exposure alone. These workers concluded that inhalation of diesel exhaust can enhance airway hyperresponsiveness and airway inflammation caused by OA sensitization in mice.

The effects of diesel exhaust on IgE antibody production were investigated in BALB/c mice sensitized with OA and exposed by inhalation to diesel exhaust (3.0 and 6.0 mg/m^3) for 3 weeks (Fujimaki et al., 1997). The mice were sensitized by intranasal administration of OA alone before, immediately after, and 3 weeks after diesel exhaust inhalation. While body and thymus weights were unchanged in the diesel exhaust-exposed and control mice, spleen weights in mice exposed to 6 mg/m^3 diesel exhaust increased significantly. Anti-OA IgE antibody titers in the sera of mice exposed to 6 mg/m^3 diesel exhaust were significantly higher than control. Total IgE and anti-OA IgG in sera from diesel exhaust-exposed and control mice remained unchanged.

Cytokine production was measured in vitro stimulated with OA in spleen cells from mice exposed to diesel exhaust (6 mg/m^3). Antigen-stimulated interleukin-4 (IL-4) and -10 (IL-10) production increased significantly in vitro in spleen cells from diesel exhaust-exposed mice compared with controls, while IFN- production decreased markedly. The authors concluded that diesel exhaust inhalation in mice may affect antigen-specific IgE antibody production through alteration of the cytokine network.

5.1.2.3.6. ***Effects on the immune system—noninhalation studies.*** The immune response of laboratory animals to DPM has been studied in various noninhalation models, and the results of these studies are presented in Table 5-9. Takafuji et al. (1987) evaluated the IgE antibody response of mice inoculated intranasally at intervals of 3 weeks with varying doses of a suspension of DPM in ovalbumin. Antiovalbumin IgE antibody titers, assayed by passive cutaneous anaphylaxis, were enhanced by doses as low as 1 μg of particles compared with immunization with ovalbumin alone.

Muranaka et al. (1986) studied the effects of DPM on IgE antibody production in immunized mice. A greater IgE antibody response was noted in mice immunized by ip injection of ovalbumin (OA) mixed with DPM than in animals immunized with OA alone. This effect of DPM on IgE antibody production in mice was also demonstrated in mice immunized with repeated injections of dinitrophenylated-OA. Moreover, a persistent IgE-antibody response to Japanese cedar pollen (JCPA), a common pollen allergen causing allergic rhinitis in Japan, was observed in mice immunized with JCPA mixed with DPM but not in animals immunized with JCPA alone. The results suggest an association between the adjuvant activity of DPM and allergic rhinitis caused by JCPA.

The potential role of oxygen radicals in injury caused by DPM was investigated by Sagai et al. (1996). These workers reported that repeated intratracheal instillation of DPM (once/week for 16 weeks) in mice caused marked infiltration of inflammatory cells, proliferation of goblet cells, increased mucus secretion, respiratory resistance, and airway constriction. Eosinophils in the submucosa of the proximal bronchi and medium bronchioles increased eightfold following instillation. Eosinophil infiltration was significantly suppressed by pretreatment with polyethyleneglycol-conjugated superoxide dismutase (PEG-SOD). Bound sialic acid concentrations in bronchial alveolar lavage fluids, an index of mucus secretion, increased with DPM, but were also suppressed by pretreatment with PEG-SOD. Goblet cell hyperplasia, airway narrowing, and airway constriction also were observed with DPM.

Respiratory resistance to acetylcholine in the DPM group was 11 times higher than in controls, and the increased resistance was significantly suppressed by PEG-SOD pretreatment.

These findings indicate that oxygen radicals caused by intratracheally instilled DPM elicit responses characteristic of bronchial asthma.

Potential adjuvant effects of DPM on the response to the model allergen OA were investigated in BALB/c mice using the popliteal lymph node (PLN) assay (Løvik et al., 1997). DPM inoculated together with OA into one hind footpad gave a significantly augmented response (increase in weight, cell numbers, and cell proliferation) in the draining popliteal lymph node as compared to DPM or OA alone. The duration of the local lymph node response was also longer when DPM was given with the allergen. The lymph node response appeared to be of a specific immunologic character and not an unspecific inflammatory reaction. The OA-specific response IgE was increased in mice receiving OA together with DPM as compared with the response in mice receiving OA alone. Further studies using carbon black (CB) as a surrogate for the nonextractable core of DPM found that while CB resembled DPM in its capacity to increase the local lymph node response and serum-specific IgE response to OA, CB appeared to be slightly less potent than DPM. The results indicate that the nonextractable particle core contributes substantially to the adjuvant activity of DPM.

Nilsen et al. (1997) investigated which part of the particle was responsible, the carbon core and/or the adsorbed organic substances, for the adjuvant activity of DPM. Female BALB/cA mice were immunized with OA alone or in combination with DPM or CB particles by intranasal administration. There was an increased response to the antigen in animals receiving OA together with DPM or CB, compared with animals receiving OA alone. The response was seen as both an increased number of responding animals and increased serum anti OA IgE response. The workers concluded that both DPM and CB have an adjuvant activity for specific IgE production, but that the activity of DPM may be more pronounced than that of CB. The results suggest that both the organic matter adsorbed to DPM and the nonextractable carbon are responsible for the observed adjuvant effect.

Fujimaki et al. (1994) investigated the relationship between DPM and IgE antibody production, interleukin 4 (IL-4) production in BALB/c mice treated with DPM mixed with antigen OA or JCPA by intratracheal instillation. BALB/c mice were injected with DPM plus OA or OA alone and, after the last instillation, the proliferative response and lymphokine production by mediastinal lymph node cells (LNC) were examined in vitro. The proliferative response to OA in mediastinal LNC from mice injected with DPM plus OA was enhanced to 4-17 times that of control mice. IL-4 production by OA stimulation was also enhanced in mediastinal LNC from mice injected with DPM plus OA. A significantly larger amount of anti-OA IgE antibody was detected in sera from DPM- and OA-injected mice compared with those from control mice. The levels of IL-4, estimated by JCP antigen in mediastinal LNC, from mice injected with DPM plus JCPA were twofold higher than those from mice injected with JCP alone. These results suggest

that intratracheal instillation of DPM affects antigen-specific IgE antibody responses via local T-cell activation, especially enhanced IL-4 production.

Suzuki et al. (1993) investigated the adjuvant activity of pyrene, a compound contained in DPM, on IgE antibody production in mice. In the first experiment, mice were immunized with 1 μg of OA alone, 1 μg of OA plus 1 mg of pyrene, or 1 μg of OA plus 1 mg of DPM, respectively. The IgE antibody responses to OA in mice immunized with OA plus pyrene or OA plus DPM were enhanced as compared to those in mice immunized with OA alone; the highest responses were observed in mice immunized with OA plus DPM. In the second experiment, mice were immunized with 10 μg of JCPA alone or 10 μg of JCPA plus 5 mg of pyrene. The IgE antibody responses to JCPA in mice immunized with JCPA plus pyrene were higher than those in mice immunized with JCPA alone. The results indicate that pyrene contained in DPM acts as an adjuvant in IgE antibody production in immunized mice.

Suzuki et al. (1996) investigated the effect of pyrene on IgE and IgG1 antibody production in mice to clarify the relation between mite allergy and adjuvancy of the chemical compounds in DPM. The mite allergen was Der f II, one of the major allergens of house dust mite (*Dermatophagoides farinae*). Allergen mice were grouped and immunized with Der f II (5 μg), Der f II (5 μg) plus pyrene (200 μg), and Der f II (5 μg) plus DPM (100 μg) intranasally seven times at 2-week intervals. The separate groups of mice were also immunized with Der f II (10 μg) plus the same dose of adjuvants in the same way. The IgE antibody responses to Der f II in mice immunized with Der f II plus pyrene or Der f II plus DPM were markedly enhanced compared with those immunized with Der f II alone. The anti-Der f II IgE antibody production increased with increasing the dose of Der f II from 5 μg to 10 μg in mice immunized with Der f II plus the same dose of adjuvants. The IgG1 antibody responses to Der f II in mice immunized with Der f II (10 μg) plus pyrene (200 μg) or Der f II (10 μg) plus DPM (100 μg) were greater than those immunized with 10 μg of Der f II alone. In addition, when peritoneal macrophages obtained from normal mice were incubated with pyrene or DPM in vitro, an enhanced IL-1a production by the macrophages was observed. When spleen lymphocytes obtained from the mice immunized with Der f II (10 μg) plus DPM (100 μg) or Der f II (10 μg) plus pyrene (200 μg) were stimulated with 10 μg of Der f II in vitro, an enhanced IL-4 production of the lymphocytes was also observed compared with those immunized with Der f II alone. This study indicates that DPM and pyrene have an adjuvant activity on IgE and IgG1 antibody production in mice immunized intranasally with a house dust mite allergen.

Maejima et al. (1997) examined the potential adjuvant activity of several different fine particles. These workers administered 25 μg of each of 5 particles (Kanto loam dust, fly ash, CB, DPM, and aluminum hydroxide [alum]) intranasally in mice and exposed them to aerosolized

JCPA for intervals up to 18 weeks. Measurements were made of JCPA-specific IgE and IgG antibody titers, the protein-adsorbing capacity of each type of particle, and nasal rubbing movements (a parameter of allergic rhinitis in mice). The increases in anti-JPCA IgE and IgG antibody titers were significantly greater in mice treated with particles and aerosolized JCPA than in mice treated with aerosolized JCPA alone. In a subsequent experiment, the mice received the particles as before, but about 160,000 grains of JCP were dropped onto the tip of the nose of each mouse twice a week for 16 weeks. After 18 weeks there were no significant differences in the anti-JCPA IgE and IgG production, nasal rubbing, or histopathological changes. The workers concluded that the nature of the particle, the ability of the particle to absorb antigens, and particle size are not related to the enhancement of IgE antibody production or symptoms of allergic rhinitis. However, IgE antibody production did appear to occur earlier in mice treated with particles than in mice immunized with allergens alone.

Eosinophils are major components of allergic inflammatory disorders including asthma and nasal allergy. Terada et al. (1997) examined the effects of DPM and DPM extract on eosinophil adhesion, survival rate, and degranulation. Eosinophils, human mucosal microvascular endothelial cells (HMMECs), and human nasal epithelial cells (HNECs) were preincubated in the presence of DPM and DPM extract. 35S-labeled eosinophils were allowed to adhere to monolayers of HMMECs and HNECs. Although neither DPM nor DPM extract affected the adhesiveness of HMMECs and HNECs to eosinophils, DPM and DPM extract each significantly increased eosinophil adhesiveness to HNECs; neither affected eosinophil adhesiveness to HMMECs. DPM extract also induced eosinophil degranulation without changing the eosinophil survival rate. These results indicate that DPM may play an important role in promoting the nasal hypersensitivity induced by enhanced eosinophil infiltration of epithelium and eosinophil degranulation.

Histamine is the most important chemical mediator in the pathogenesis of nasal allergy. Terada et al. (1999) examined the effects of DPM extract on the expression of histamine H1 receptor (H1R) mRNA in HNECs and HMMECs, and on the production of IL-8 and GM-CSF induced by histamine. HNECs and HMMECs, isolated from human nasal mucosa specimens, were cultured with DPM extract. DPM extract increased the expression of H1R mRNA in both HNECs and HMMECs. The amount of IL-8 and GM-CSF, induced by histamine, was also significantly higher in HNECs and HMMECs treated with DPM extract. These results strongly suggest that DPM accelerates the inflammatory change by not only directly upregulating H1R expression but also by increasing histamine-induced IL-8 and GM-CSF production.

The potential for DPM to modulate cytokine production has been demonstrated in cultured mouse bone marrow-derived mast cells (BMMC). Saneyoshi et al. (1997) examined the production of cytokines in BMMC treated with DPM (0.8, 2 and 4 μg/mL). Production of interleukin-4 (IL-4) and IL-6 was higher in BMMC stimulated with A23187 and treated with low

concentrations of DPM than in controls, but no increase was seen in BMMC treated with high DPM. After pretreatment with low DPM for 24 h, IL-4 production in BMMC stimulated with A23187 was lower than in controls. Antigen-induced IL-4 production increased significantly in BMMC treated with 0.4 or 0.8 μg/mL DPM, but did not increase with low DPM. Although the enhancement of IL-4 production of BMMC stimulated with A23187 plus DPM was not completely inhibited by 2-mercaptoethanol, treatment with dexamethasone inhibited further IL-4 production. Thus, DPM may affect the immune response via the modulation of cytokine production in mast cells.

Ormstad et al. (1998) investigated the potential for DPM as well as other suspended particulate matter (SPM) to act as a carrier for allergens into the airways. These investigators found both Can f 1 (dog) and Bet v 1 (birch pollen) on the surface of SPM collected in air from different homes. In an extension of the study, they found that DPM had the potential of binding, in vitro, both of these allergens as well as Fel d 1 (cat) and Der p 1 (house mite). The authors conclude that soot particles in indoor air house dust may act as carrier of several allergens in indoor air.

Knox et al. (1997) investigated whether free grass pollen allergen molecules, released from pollen grains by osmotic shock (Suphioglu et al., 1992) and dispersed in microdroplets of water in aerosols, can bind to DPM in air. Using natural highly purified Lol p 1, immunogold labeling with specific monoclonal antibodies, and a high-voltage transmission electron-microscopic imaging technique, these workers demonstrated binding of the major grass pollen allergen, Lol p 1, to DPM in vitro. These workers conclude that binding of DPM with Lol p 1 might be a mechanism by which allergens can become concentrated in air and trigger attacks of asthma.

The inhalation of diesel exhaust appeared to have minimal effects on the immune status of rats and guinea pigs. Conversely, intranasally delivered doses as low as 1 μg of DPM exerted an adjuvant activity for IgE antibody production in mice. Further studies of the effects of diesel exhaust on the immune system are needed to clarify the impact of such variables as route of exposure, species, dose, and atopy.

Murphy et al. (1999) examined the comparative toxicities to the lung of four CB particles and DPM, in primary cultures of mouse Clara and rat type II epithelial cells. Particle toxicity was assessed by cell attachment to an extracellular matrix substratum. The CB particles varied in toxicity to Clara and type II cells. DPM stored for 2 weeks was equally toxic to both cell types. DPM became progressively less toxic to type II cells with time of storage. Both primary epithelial cell types internalized the particles in culture. These workers concluded that bioreactivity was related to CB particle size and surface area, with the smaller particles having the larger surface

area being the more toxic. Although freshly prepared DPM was equally toxic to type II and Clara cells, DPM became progressively less toxic to the type II cells with time.

Exposure studies in laboratory animals and isolated cell systems derived from animals also indicate that DPM can elicit both inflammatory and immunological changes. Moreover, the effects appear to be due to both the nonextractable carbon core and the adsorbed organic fraction of the diesel particle. The data further indicate a role for oxygen radicals in DPM injury because the extent of the injury can be reduced by treatment with antioxidants. DPM also has the capacity to bind and transport airborne allergens.

5.1.2.3.7. ***Effects on the liver***. Meiss et al. (1981) examined alterations in the hepatic parenchyma of hamsters by using thin-section and freeze-fracture histological techniques. Exposures to diesel exhaust were for 7 to 8 h/day, 5 days/week, for 5 mo at about 4 or 11 mg/m^3 DPM. The livers of the hamsters exposed to both concentrations of diesel exhaust exhibited moderate dilatation of the sinusoids, with activation of the Kupffer cells and slight changes in the cell nuclei. Fatty deposits were observed in the sinusoids, and small fat droplets were occasionally observed in the peripheral hepatocytes. Mitochondria often had a loss of cristae and exhibited a pleomorphic character. Giant microbodies were seen in the hepatocytes, which were moderately enlarged, and gap junctions between hepatocytes exhibited a wide range in structural diversity. The results of this study and others on the effect of exposure of diesel exhaust on the liver of laboratory animals are summarized in Table 5-10.

Green et al. (1983) and Plopper et al. (1983) reported no changes in liver weights of rats exposed to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 52 weeks or of cats exposed to 6 to 12 mg/m^3, 8 h/day, 7 days/week for 124 weeks. The use of light and electron microscopy revealed that long-term inhalation of varying high concentrations of diesel exhaust caused numerous alterations to the hepatic parenchyma of guinea pigs. A less sensitive index of liver toxicity, increased liver weight, failed to detect an effect of diesel exhaust on the liver of the rat and cat following long-term exposure to diesel exhaust. These results are too limited to understand potential impacts on the liver.

5.1.2.3.8. ***Blood and cardiovascular systems.*** Several studies have evaluated the effects of diesel exhaust exposure on hematological and cardiovascular parameters of laboratory animals. These studies are summarized in Table 5-11. Standard hematological indices of toxicological effects on red and white blood cells failed to detect dramatic and consistent responses. Erythrocyte (RBC) counts were reported as being unaffected in cats (Pepelko and Peirano, 1983), rats and monkeys (Lewis et al., 1989), guinea pigs and rats (Penney et al., 1981), and rats (Karagianes et al., 1981); lowered in rats (Heinrich et al., 1982); and elevated in rats (Research

Committee for HERP Studies, 1988; Brightwell et al., 1986). Mean corpuscular volume was significantly increased in monkeys, 69 versus 64 (Lewis et al., 1989), and hamsters (Heinrich et al., 1982), and lowered in rats (Research Committee for HERP Studies, 1988). The only other parameters of erythrocyte status and related events were lowered mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in rats (Research Committee for HERP Studies, 1988), a 3% to 5% increase in carboxyhemoglobin saturation in rats (Karagianes et al., 1981), and a suggestion of an increase in prothrombin time (Brightwell et al., 1986). The biological significance of these findings regarding adverse health effects is deemed to be inconsequential.

Three investigators (Pepelko and Peirano, 1983; Lewis et al., 1989; Brightwell et al., 1986) reported an increase in the percentage of banded neutrophils in cats and rats. This effect was not observed in monkeys (Lewis et al., 1989). The health implications of an increase in abnormal maturation of circulating neutrophils are uncertain but indicate a toxic response of leukocytes following exposures to diesel exhaust. Leukocyte counts were reported to be reduced in hamsters (Heinrich et al., 1982); increased in rats (Brightwell et al., 1986); and unaffected in cats, rats, and monkeys (Pepelko and Peirano, 1983; Research Committee for HERP Studies, 1988; Lewis et al., 1989). These inconsistent findings indicate that the leukocyte counts are more indicative of the clinical status of the laboratory animals than any direct effect of exposure to diesel exhaust.

No significant changes in heart mass were found in guinea pigs or rats exposed to diesel exhaust (Wiester et al., 1980; Penney et al., 1981; Lewis et al., 1989). Rats exposed to diesel exhaust showed a greater increase in the medial wall thickness of pulmonary arteries of differing diameters and right ventricular wall thickness; these increases, however, did not achieve statistically significant levels (Vallyathan et al., 1986). Brightwell et al. (1986) reported increased heart/body weight and right ventricular/heart weight ratios and decreased left ventricular contractility in rats exposed to 6.6 mg/m^3 DPM for 16 h/day, 5 days/week for 104 weeks.

The effects of DPM on the endothelium-dependent relaxation (EDR) of vascular smooth muscle cells have been investigated (Ikeda et al., 1995, 1998). Incubation of rat thoracic aortae with suspensions of DPM (10-100 μg/mL) markedly attenuated acetylcholine-induced EDR. The mechanism of this effect was studied further in cultured porcine endothelial cells (CPE). A 10-min incubation of PEC with DPM (0.1-100 μg/mL) inhibited endothelium-dependent relaxing factor (EDRF) or nitric oxide (NO) release. A 10-min incubation of DPM with NO synthase inhibited formation of NO_2^-, a product of NO metabolism. The authors concluded that DPM, at the concentrations tested, neither induced cell damage nor inhibited EDRF release from PEC, but scavenged and thereby blocked the physiological action of NO.

5.1.2.3.9. *Serum chemistry*. A number of investigators have studied the effects of exposure to diesel exhaust on serum biochemistry, and no consistent effects have been found. Such studies are summarized in Table 5-12.

The biological significance of changes in serum chemistry in female but not male rats exposed at 2 mg/m^3 DPM for 7 h/day, 5 days/week for 104 weeks (Lewis et al., 1989) is difficult to interpret. Not only were the effects noted in one sex (females) only, but the serum enzymes, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT), were elevated in the control group, a circumstance contrary to denoting organ damage in the exposed female rats. The elevations of liver-related serum enzymes in the control versus the exposed female rats appear to be a random event among these aged subjects. The incidence of age-related disease, such as mononuclear cell leukemia, can markedly affect such enzyme levels, seriously compromising the usefulness of a comparison to historical controls. The serum sodium values of 144 versus 148 mmol/L in control and exposed rats, respectively, although statistically different, would have no biological import.

The increased serum enzyme activities, alkaline phosphatase, SGOT, SGPT, gamma-glutamyl transpeptidase, and decreased cholinesterase activity suggest an impaired liver; however, such an impairment was not established histopathologically (Heinrich et al., 1982; Research Committee for HERP Studies, 1988; Brightwell et al., 1986). The increased urea nitrogen, electrolyte levels, and gamma globulin concentration and reduction in total blood proteins are indicative of impaired kidney function. Again, there was no histopathological confirmation of impaired kidneys in these studies.

Clinical chemistry studies suggest impairment of both liver and kidney functions in rats and hamsters chronically exposed to high concentrations of diesel exhaust. The absence of histopathological confirmation, the appearance of such effects near the end of the lifespan of the laboratory animal, and the failure to find such biochemical changes in cats exposed to a higher dose, however, tend to discredit the probability of hepatic and renal hazards to humans exposed at atmospheric levels of diesel exhaust.

5.1.2.3.10. *Effects on microsomal enzymes*. Several studies have examined the effects of diesel exhaust exposure on microsomal enzymes associated with the metabolism and possible activation of xenobiotics, especially polynuclear aromatic hydrocarbons. These studies are summarized in Table 5-13. Lee et al. (1980) measured the activities of aryl hydrocarbon hydroxylase (AHH) and epoxide hydrase (EH) in liver, lung, testis, and prostate gland of adult male rats exposed to 6.32 mg/m^3 DPM 20 h/day for 42 days. Maximal significant AHH activities (pmol/min/mg microsomal protein) occurred at different times during the exposure period, and differences between controls and exposed rats, respectively, were as follows: prostate 0.29 versus 1.31, lung 3.67 versus 5.11,

and liver 113.9 versus 164.0. There was no difference in AHH activity in the testis between exposed and control rats. Epoxide hydrase activity was not significantly different from control values for any of the organs tested.

Pepelko and Peirano (1983) found no statistically significant differences in liver microsomal cytochrome P448-450 levels and liver microsomal AHH between control and diesel-exposed mice at either 6 or 8 mo of exposure. Small differences were noted in the lung microsomal AHH activities, but these were believed to be artifactual differences, due to increases in nonmicrosomal lung protein present in the microsomal preparations. Exposures to 6 mg/m^3 DPM were for 8 h/day, 7 days/week.

Rabovsky et al. (1984) investigated the effect of chronic exposure to diesel exhaust on microsomal cytochrome P450-associated benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin deethylase activities in rat lung and liver. Male rats were exposed for 7 h/day, 5 days/week for 104 weeks to 2 mg/m^3 DPM. The exposure had no effect on B[*a*]P hydroxylase or 7-ethoxycoumarin deethylase activities in lung or liver. In related studies, Rabovsky et al. (1986) examined the effects of diesel exhaust on vitally induced enzyme activity and interferon production in female mice. The mice were exposed for 7 h/day, 5 days/week for 1 mo to diesel exhaust diluted to achieve a concentration of 2 mg/m^3 DPM. After the exposure, the mice were inoculated intranasally with influenza virus. Changes in serum levels of interferon and liver microsomal activities of 7-ethoxycoumarin, ethylmorphine demethylase, and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome c reductase were measured. In the absence of viral inoculation, exposure to diesel exhaust had no significant effects on the activity levels of the two liver microsomal monooxygenases and NADPH-dependent cytochrome c reductase. Exposure to diesel exhaust produced smaller increases in ethylmorphine demethylase activity on days 2 to 4 postvirus infection and also abolished the day 4 postinfection increase in NADPH-dependent cytochrome c reductase when compared with nonexposed mice. These data suggested to the authors that the relationship that exists between metabolic detoxification and resistance to infection in unexposed mice was altered during a short-term exposure to diesel exhaust.

Chen and Vostal (1981) measured the activity of AHH and the content of cytochrome P450 in the lungs and livers of rats exposed by inhalation or intraperitoneal (i.p.) injection of a dichloromethane extract of DPM. In the inhalation exposures, the exhaust was diluted to achieve concentrations of 0.75 or 1.5 mg/m^3 DPM, and the exposure regimen was 20 h/day, 5.5 days/week for up to 9 mo. The concentration of total hydrocarbons and particle-phase hydrocarbons was not reported. Parenteral administration involved repeated i.p. injections at several dose levels for 4 days. Inhalation exposure had no significant effect on liver microsomal AHH activity; however, lung AHH activity was slightly reduced after 6 mo exposure to

1.5 mg/m^3. An i.p. dose of DPM extract, estimated to be equivalent to the inhalation exposure, had no effect on AHH activity in liver or lungs. No changes were observed in cytochrome P450 contents in lungs or liver following inhalation exposure or i.p. treatment. Direct intratracheal administration of a dichloromethane DPM extract required doses greater than 6 mg/kg body weight before the activity of induced AHH in the lung was barely doubled; liver AHH activity remained unchanged (Chen, 1986).

In related studies, Navarro et al. (1981) evaluated the effect of exposure to diesel exhaust on rat hepatic and pulmonary microsomal enzyme activities. The same exposure regimen was employed (20 h/day, 5.5 days/week, for up to 1 year), and the exhaust was diluted to achieve concentrations of 0.25 and 1.5 mg/m^3 DPM (a few studies were also conducted at 0.75 mg/m^3). After 8 weeks of exposure, there was no evidence for the induction of cytochrome P450, cytochrome P448, or NADPH-dependent cytochrome c reductase in rat liver microsomes. One year of exposure had little, if any, effect on the hepatic metabolism of B[*a*]P. However, 1 year of exposure to 0.25 and 1.5 mg/m^3 significantly impaired the ability of lung microsomes to metabolize B[*a*]P (0.15 and 0.02 nmole/30 min/mg protein, respectively, versus 0.32 nmole/30 min/mg protein for the controls).

There are conflicting results regarding the induction of microsomal AHH activities in the lungs and liver of rodents exposed to diesel exhaust. One study reported induced AHH activity in the lungs, liver, and prostate of rats exposed to diesel exhaust containing 6.32 mg/m^3 DPM for 20 h/day for 42 days; however, no induction of AHH was observed in the lungs of rats and mice exposed to 6 mg/m^3 DPM for 8 h/day, 7 days/week for up to 8 mo or to 0.25 to 2 mg/m^3 for periods up to 2 years. Exposure to diesel exhaust has not been shown to produce adverse effects on microsomal cytochrome P450 in the lungs or liver of rats or mice. The weight of evidence suggests that the absence of enzyme induction in the rodent lung exposed to diesel exhaust is caused either by the unavailability of the adsorbed hydrocarbons or by their presence in insufficient quantities for enzyme induction.

5.1.2.3.11. ***Effects on behavior and neurophysiology***. Studies on the effects of exposure to diesel exhaust on the behavior and neurophysiology of laboratory animals are summarized in Table 5-14. Laurie et al. (1978) and Laurie et al. (1980) examined behavioral alterations in adult and neonatal rats exposed to diesel exhaust. Exposure for 20 h/day, 7 days/week, for 6 weeks to exhaust containing 6 mg/m^3 DPM produced a significant reduction in adult spontaneous locomotor activity (SLA) and in neonatal pivoting (Laurie et al., 1978). In a follow-up study, Laurie et al. (1980) found that shorter exposure (8 h/day) to 6 mg/m^3 DPM also resulted in a reduction of SLA in adult rats. Laurie et al. (1980) conducted additional behavioral tests on adult rats exposed during their neonatal period. For two of three exposure situations (20 h/day for

17 days postparturition, or 8 h/day for the first 28 or 42 days postparturition), significantly lower SLA was observed in the majority of the tests conducted on the adults after week 5 of measurement. When compared with control rats, adult 15-month-old rats that had been exposed as neonates (20 h/day for 17 days) also exhibited a significantly slower rate of acquisition of a bar-pressing task to obtain food. The investigators noted that the evidence was insufficient to determine whether the differences were the result of a learning deficit or due to some other cause (e.g., motivational or arousal differences).

These data are difficult to interpret in terms of health hazards to humans under ambient environmental conditions because of the high concentration of diesel exhaust to which the laboratory rats were exposed. Additionally, there are no further concentration-response studies to assess at what exposure levels these observed results persist or abate. A permanent alteration in both learning ability and activity resulting from exposures early in life is a health hazard whose significance to humans should be pursued further.

Neurophysiological effects from exposure to diesel exhaust were investigated in rats by Laurie and Boyes (1980, 1981). Rats were exposed to diluted diesel exhaust containing 6 mg/m^3 DPM for 8 h/day, 7 days/week from birth up until 28 days of age. Somatosensory evoked potential, as elicited by a 1 mA electrical pulse to the tibial nerve in the left hind limb, and visual evoked potential, as elicited by a flash of light, were the endpoints tested. An increased pulse latency was reported for the rats exposed to diesel exhaust, and this was thought to be caused by a reduction in the degree of nerve myelinization. There was no neuropathological examination, however, to confirm this supposition.

Based on the data presented, it is not possible to specify the particular neurological impairment(s) induced by the exposure to diesel exhaust. Again, these results occurred following exposure to a high level of diesel exhaust and no additional concentration-response studies were performed.

5.1.2.3.12. ***Effects on reproduction and development***. Studies of the effects of exposure to diesel exhaust on reproduction and development are summarized in Table 5-15. Twenty rats were exposed 8 h/day on days 6 through 15 of gestation to diluted diesel exhaust containing 6 mg/m^3 DPM (Werchowski et al., 1980a,b; Pepelko and Peirano, 1983). There were no signs of maternal toxicity or decreased fertility. No skeletal or visceral teratogenic effects were observed in 20-day-old fetuses (Werchowski et al., 1980a). In a second study, 42 rabbits were exposed to 6 mg/m^3 DPM for 8 h/day on gestation days 6 through 18. No adverse effects on body weight gain or fertility were seen in the does exposed to diesel exhaust. No visceral or skeletal developmental abnormalities were observed in the fetuses (Werchowski et al., 1980b).

Pepelko and Peirano (1983) evaluated the potential for diesel exhaust to affect reproductive performance in mice exposed from 100 days prior to exposure throughout maturity of the F_2 generation. The mice were exposed for 8 h/day, 7 days/week to 12 mg/m^3 DPM. In general, treatment-related effects were minimal. Some differences in organ and body weights were noted, but overall fertility and survival rates were not altered by exposure to diesel exhaust. The only consistent change, an increase in lung weights, was accompanied by a gross pathological diagnosis of anthracosis. These data denoted that exposure to diesel exhaust at a concentration of 12 mg/m^3 did not affect reproduction. See Section 5.3, which reports a lack of effects of exposure to diesel exhaust on rat lung development (Mauderly et al., 1987b).

Several studies have evaluated the effect of exposure to diesel exhaust on sperm. Lewis et al. (1989) found no adverse sperm effects (sperm motility, velocity, densities, morphology, or incidence of abnormal sperm) in monkeys exposed for 7 h/day, 5 days/week for 104 weeks to 2 mg/m^3 DPM. In another study in which A/Strong mice were exposed to diesel exhaust containing 6 mg/m^3 DPM for 8 h/day for 31 or 38 weeks, no significant differences were observed in sperm morphology between exposed and control mice (Pereira et al., 1981). It was noted, however, that there was a high rate of spontaneous sperm abnormalities in this strain of mice, and this may have masked any small positive effect. Quinto and De Marinis (1984) reported a statistically significant and dose-related increase in sperm abnormalities in mice injected intraperitoneally for 5 days with 50, 100, or 200 mg/kg of DPM suspended in corn oil. A significant decrease in sperm number was seen at the highest dose, but testicular weight was unaffected by the treatment.

Watanabe and Oonuki (1999) investigated the effects of diesel engine exhaust on reproductive endocrine function in growing rats. The rats were exposed to whole diesel engine exhaust (5.63 mg/m^3 DPM, 4.10 ppm NO_2, and 8.10 ppm NO_x); a group was exposed to filtered exhaust without DPM, and a group was exposed to clean air. Exposures were for 3 mo beginning at birth (6 h/day for 5 days/week).

Serum levels of testosterone and estradiol were significantly higher and follicle-stimulating hormone significantly lower in animals exposed to whole diesel exhaust and filtered exhaust compared to controls. Luteinizing hormone was significantly decreased in the whole-exhaust-exposed group as compared to the control and filtered groups. Sperm production and activity of testicular hyaluronidase were significantly reduced in both exhaust-exposed groups as compared to the control group. This study suggests that diesel exhaust stimulates hormonal secretion of the adrenal cortex, depresses gonadotropin-releasing hormone, and inhibits spermatogenesis in rats. Because these effects were not inhibited by filtration, the gaseous phase of the exhaust appears more responsible than particulate matter for disrupting the endocrine system.

No teratogenic, embryotoxic, fetotoxic, or female reproductive effects were observed in mice, rats, or rabbits at exposure levels up to 12 mg/m^3 DPM. Effects on sperm morphology and

number were reported in hamsters and mice exposed to high doses of DPM; however, no adverse effects were observed in sperm obtained from monkeys exposed at 2 mg/m^3 for 7 h/day, 5 days/week for 104 weeks. Concentrations of 12 mg/m^3 DPM did not affect male rat reproductive fertility in the F_0 and F_1 generation breeders. Thus, exposure to diesel exhaust would not appear to be a reproductive or developmental hazard.

5.2. MODE OF ACTION OF DIESEL EMISSIONS-INDUCED NONCANCER EFFECTS

5.2.1. Comparison of Health Effects of Filtered and Unfiltered Diesel Exhaust

In four chronic toxicity studies of diesel exhaust, the experimental protocol included exposing test animals to exhaust containing no particles. Comparisons were then made between the effects caused by whole, unfiltered exhaust and those caused by the gaseous components of the exhaust. Concentrations of components of the exposure atmospheres in these four studies are given in Table 5-16.

Heinrich et al. (1982) compared the toxic effects of whole and filtered diesel exhaust on hamsters and rats. Exposures were for 7 to 8 h/day and 5 days/week. Rats exposed for 24 mo to either whole or filtered exhaust exhibited no significant changes in respiratory frequency, respiratory minute volume, compliance or resistance as measured by a whole-body plethysmography, or heart rate. In the hamsters, histological changes (adenomatous proliferations) were seen in the lungs of animals exposed to either whole or filtered exhaust; however, in all groups exposed to the whole exhaust the number of hamsters exhibiting such lesions was significantly higher than for the corresponding groups exposed to filtered exhaust or clean air. Severity of the lesions was, however, not reported.

In a second study, Heinrich et al. (1986a, see also Stöber, 1986) compared the toxic effects of whole and filtered diesel exhaust on hamsters, rats, and mice. The test animals (96 per test group) were exposed for 19 h/day, 5 days/week for 120 (hamsters and mice) or 140 (rats) weeks. Body weights of hamsters were unaffected by either exposure. Body weights of rats and mice were reduced by the whole exhaust but not by the filtered exhaust. Exposure-related higher mortality rates occurred in mice after 2 years of exposure to whole exhaust. After 1 year of exposure to the whole exhaust, hamsters exhibited increased lung weights, a significant increase in airway resistance, and a nonsignificant reduction in lung compliance. For the same time period, rats exhibited increased lung weights, a significant decrease in dynamic lung compliance, and a significant increase in airway resistance. Test animals exposed to filtered exhaust did not exhibit such effects. Histopathological examination indicated that different levels of response occurred in the three species. In hamsters, filtered exhaust caused no significant histopathological effects in the lung; whole exhaust caused thickened alveolar septa, bronchioloalveolar hyperplasia, and

emphysematous lesions. In mice, whole exhaust, but not filtered exhaust, caused multifocal bronchioloalveolar hyperplasia, multifocal alveolar lipoproteinosis, and multifocal interstitial fibrosis. In rats, there were no significant morphological changes in the lungs following exposure to filtered exhaust. In rats exposed to whole exhaust, there were severe inflammatory changes in the lungs, thickened alveolar septa, foci of macrophages, crystals of cholesterol, and hyperplastic and metaplastic lesions. Biochemical studies of lung lavage fluids of hamsters and mice indicated that exposure to filtered exhaust caused fewer changes than did exposure to whole exhaust. The latter produced significant increases in lactate dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, total protein, protease (pH 5.1), and collagen. The filtered exhaust had a slight but nonsignificant effect on G6P-DH, total protein, and collagen. Similarly, cytological studies showed that while the filtered exhaust had no effect on differential cell counts, the whole exhaust resulted in an increase in leukocytes (161 ± 43.3/μL versus 55.7 ± 12.8/μL in the controls), a decrease in AMs (30.0 ± 12.5 versus 51.3 ± 12.5/μL in the controls), and an increase in granulocytes (125 ± 39.7 versus 1.23 ± 1.14/μL in the controls). All values presented for this study are the mean with its standard deviation. The differences were significant for each cell type. There was also a small increase in lymphocytes (5.81 ± 4.72 versus 3.01 ± 1.23/μL in the controls).

Iwai et al. (1986) exposed rats (24 per group) to whole or filtered diesel exhaust 8 h/day, 7 days/week for 24 mo. The whole exhaust was diluted to achieve a concentration of 4.9 ± 1.6 mg/m^3 DPM. Body weights in the whole exhaust group began to decrease after 6 mo and in both exposed groups began to decrease after 18 mo when compared with controls. Lung-to-body weight ratios of the rats exposed to the whole exhaust showed a significant increase (p<0.01) after 12 mo in comparison with control values. Spleen-to-body weight ratios of both exposed groups were higher than control values after 24 mo. After 6 mo of exposure to whole exhaust, DPM accumulated in AMs, and Type II cell hyperplasia was observed. After 2 years of exposure, the alveolar walls had become fibrotic with mast cell infiltration and epithelial hyperplasia. In rats exposed to filtered exhaust, after 2 years there were only minimal histologic changes in the lungs, with slight hyperplasia and stratification of bronchiolar epithelium and infiltration of atypical lymphocytic cells in the spleen.

Brightwell et al. (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Three exhaust dilutions were tested, producing concentrations of 0.7, 2.2, and 6.6 mg/m^3 DPM. The test animals (144 rats and 312 hamsters per exposure group) were exposed for five 16-h periods per week for 2 years. The four exposure types were gasoline, gasoline catalyst, diesel, and filtered diesel. The results presented were limited to statistically significant differences between exhaust-exposed and control animals. The inference from the discussion section of the paper was that there was a minimum of toxicity in the animals exposed to filtered

diesel exhaust: "It is clear from the results presented that statistically significant differences between exhaust-exposed and control animals are almost exclusively limited to animals exposed to either gasoline or unfiltered diesel exhaust." Additional results are described in Section 5.1.2.3.

Heinrich et al. (1995) exposed female NMRI and C57BL/6N mice to a diesel exhaust dilution that resulted in a DPM concentration of 4.5 mg/m^3 and to the same dilution after filtering to remove the particles. This study is focused on the carcinogenic effects of DPM exposure, and inadequate information was presented to compare noncancer effects in filtered versus unfiltered exhaust.

A comparison of the toxic responses in laboratory animals exposed to whole exhaust or filtered exhaust containing no particles demonstrates across studies that when the exhaust is sufficiently diluted to limit the concentrations of gaseous irritants (NO_2 and SO_2), irritant vapors (aldehydes), CO, or other systemic toxicants, the diesel particles are the prime etiologic agents of noncancer health effects, although additivity or synergism with the gases cannot be ruled out. These toxic responses are both functional and pathological and represent cascading sequelae of lung pathology based on concentration and species. The diesel particles plus gas exposures produced biochemical and cytological changes in the lung that are much more prominent than those evoked by the gas phase alone. Such marked differences between whole and filtered diesel exhaust are also evident from general toxicological indices, such as decreases in body weight and increases in lung weights, pulmonary function measurements, and pulmonary histopathology (e.g., proliferative changes in Type II cells and respiratory bronchiolar epithelium, fibrosis). Hamsters, under equivalent exposure regimens, have lower levels of retained DPM in their lungs than rats and mice do and, consequently, less pulmonary function impairment and pulmonary pathology. These differences may result from lower DPM inspiration and deposition during exposure, greater DPM clearance, or lung tissue less susceptible to the cytotoxicity of deposited DPM.

5.2.2. Mode of Action for the Noncarcinogenic Effects of DPM

As noted in Chapter 2, diesel emissions are a complex mixture that includes both a vapor phase and a particle phase. The particle phase consists of poorly soluble carbon particles on the surfaces of which are adsorbed a large number of organic and inorganic compounds. Although the effects to be discussed are considered attributable to the particle phase (termed diesel particulate matter or DPM), additive or synergistic effects due to the vapor phase cannot be totally discounted. This may be especially so in the human studies and the animal toxicology studies where exposure is to various dilutions of diesel emissions, or in the in vitro studies in which the test material was captured by filtration.

The mechanisms by which DPM is inhaled, deposited, and cleared from the respiratory tract are discussed in Chapter 3. DPM deposited upon airway surfaces may be cleared from the

respiratory tract completely, or may be translocated to other sites within the respiratory system. The pathogenic sequence following the deposition of inhaled DPM begins with the interaction of DPM with airway epithelial cells and phagocytosis by AMs. The airway epithelial cells and activated AMs release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of DPM increases, there is an aggregation of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type II cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes.

The macrophages engulfing the DPM may release cytokines, growth factors, and proteases, which may cause inflammation, cell injury, cell proliferation, hyperplasia, and fibrosis. This is especially true under lung overload conditions occurring in laboratory rats when the rate of deposition exceeds the rate of alveolar clearance. This phenomenon is described in Chapter 3. The mechanisms leading to the generation of oxygen radicals and subsequent lung injury are described in Chapter 7.

DPM is a poorly soluble particle whose rate of clearance by dissolution is insignificant compared to its rate of clearance as an intact particle. The organic material adsorbed to the surface is desorbed from the DPM and may enter into metabolic reactions and be activated and enter into reactions with other macromolecules or be detoxified and excreted (Figure 7-1). The diesel particle may be cleared directly by the clearance mechanisms described in Chapter 3.

The organic material desorbed from the particle (described in Chapter 7) appears to be associated with the immunological changes described in Section 5.1.1.1.4. The potential adjuvant effects of DPM have also been studied. The results, described in Section 5.1.2.3.6, indicate that while the nonextractable particle core contributes substantially to the adjuvant activity of DPM, the organic matter adsorbed to DPM, notably pyrene, also augments the adjuvant effect.

Thus, the available evidence indicates that DPM has the potential to produce pathological and immunological changes in the respiratory tract. Moreover, the magnitude of these responses is determined by the dose delivered to the respiratory tract and is attributable to both the carbon core and the adsorbed organic materials.

5.3. INTERACTIVE EFFECTS OF DIESEL EXHAUST

A multitude of factors may influence the susceptibility to exposure to diesel exhaust as well as the resulting response. Some of these have already been discussed in detail (e.g., the composition of diesel exhaust and concentration-response data); others will be addressed in this section (e.g., the interaction of diesel exhaust with factors particular to the exposed individual and the interaction of diesel exhaust components with other airborne contaminants).

Mauderly et al. (1990a) compared the susceptibility of normal rats and rats with preexisting laboratory-induced pulmonary emphysema exposed for 7 h/day, 5 days/week for 24 mo to diesel exhaust containing 3.5 mg/m^3 DPM or to clean air (controls). Emphysema was induced in one-half of the rats by intratracheal instillation of elastase 6 weeks before exhaust exposure. Measurements included lung burdens of DPM, respiratory function, bronchoalveolar lavage, clearance of radiolabeled particles, pulmonary immune responses, lung collagen, excised lung weight and volume, histopathology, and mean linear intercept of terminal air spaces. None of the data for the 63 parameters measured suggest that rats with emphysematous lungs were more susceptible than rats with normal lungs to the effects of diesel exhaust exposure. In fact, each of the 14 emphysema-exhaust interactions detected by statistical analysis of variance indicated that emphysema acted to reduce the effects of diesel exhaust exposure. DPM accumulated much less rapidly in the lungs of emphysematous rats than in those of normal rats. The mean lung burdens of DPM in the emphysematous rats were 39%, 36%, and 37% of the lung burdens of normal rats at 12, 18, and 24 mo, respectively. No significant interactions were observed among lung morphometric parameters. Emphysema prevented the exhaust-induced increase for three respiratory indices of expiratory flow rate at low lung volumes, reduced the exhaust-induced increase in nine lavage fluid indicators of lung damage, prevented the expression of an exhaust-induced increase in lung collagen, and reduced the exhaust-induced delay in DPM clearance.

Mauderly et al. (1987b) evaluated the relative susceptibility of developing and adult rat lungs to damage by exposure to diesel exhaust. Rats (48 per test group) were exposed to diesel exhaust containing 3.5 mg/m^3 DPM and about 0.8 ppm NO_2. Exposures were for 7 h/day, 5 days/week through gestation to the age of 6 mo, or from the age of 6 to 12 mo. Comparative studies were conducted on respiratory function, immune response, lung clearance, airway fluid enzymes, protein and cytology, lung tissue collagen, and proteinases in both age groups. After the 6-mo exposure, adult rats, compared with controls, exhibited (1) more focal aggregates of particle-containing AMs in the alveolar ducts near the terminal bronchioles, (2) a sixfold increase in the neutrophils (as a percentage of total leukocytes) in the airway fluids, (3) a significantly higher number of total lymphoid cells in the pulmonary lymph nodes, (4) delayed clearance of DPM and radiolabeled particles ($t_{1/2}$ = 90 days versus 47 days for controls), and (5) increased lung weights. These effects were not seen in the developing rats. On a weight-for-weight (milligrams of DPM per gram of lung) basis, DPM accumulation in the lungs was similar in developing and adult rats immediately after the exposure. During the 6-mo postexposure period, DPM clearance was much more rapid in the developing rats, approximately 2.5-fold. During postexposure, diesel particle-laden macrophages became aggregated in the developing rats, but these aggregations were located primarily in a subpleural position. The authors concluded that exposure to diesel

exhaust, using pulmonary function, structural (qualitative or quantitative) biochemistry as the indices, did not affect the developing rat lung more severely than the adult rat lung.

As a result of the increasing trend of using diesel-powered equipment in coal mining operations and the concern for adverse health effects in coal miners exposed to both coal dust or coal mine dust and diesel exhaust, Lewis et al. (1989) and Karagianes et al. (1981) investigated the interaction of coal dust and diesel exhaust. Lewis et al. (1989) exposed rats, mice, and cynomolgus monkeys to (1) filtered ambient air, (2) 2 mg/m^3 DPM, (3) 2 mg/m^3 respirable coal dust, and (4) 1 mg/m^3 of both DPM and respirable coal dust. Gaseous and vapor concentrations were identical in both diesel exhaust exposures. Exposures were for 7 h/day, 5 days/week for up to 24 mo. Synergistic effects between diesel exhaust and coal dust were not demonstrated; additive toxic effects were the predominant effects noted.

Karagianes et al. (1981) exposed rats (24 per group) to diesel exhaust containing 8.3 mg/m^3 of DPM alone or in combination with about 6 mg/m^3 of coal dust. No synergistic effects were found between diesel exhaust and coal dust; additive effects in terms of visual dust burdens in necropsied lungs were related to dose (i.e., length of exposure and airborne particulate concentrations).

The health effects of airborne contaminants from sources other than diesel engines may be altered in the presence of DPM by their adsorption onto the diesel particles. When adsorbed onto diesel particles, the gases and vapors can be transported and deposited deeper into the lungs, and because they are more concentrated on the particle surface, the resultant cytotoxic effects or physiological responses may be enhanced. Nitrogen dioxide adsorbed onto carbon particles caused pulmonary parenchymal lesions in mice, whereas NO_2 alone produced edema and inflammation but no lesions (Boren, 1964). Exposure to formaldehyde and acrolein adsorbed onto carbon particles (1 to 4 μm) resulted in the recruitment of PMNs to tracheal and intrapulmonary epithelial tissues but not when the aldehydes were tested alone (Kilburn and McKenzie, 1978).

Madden et al. (2000) observed that O_3 exposure increased the bioactivity of DPM. DPM, pre-exposed to O_3 for 48 h, was instilled into the lungs of laboratory rats. Lung inflammation and injury were examined 24 h after instillation by lung lavage. DPM pre-exposed to 0.1 PPM O_3 was more potent in increasing neutrophilia, lavage total protein, and LDH compared to unexposed DPM. Treatment of DPM with higher concentrations of O_3 (1.0 PPM) decreased the bioactivity of the particles.

There is no direct evidence that diesel exhaust, at concentrations found in the ambient environment, interacts with other substances in the exposure environment or the physiological status of the exposed subject other than impaired resistance to respiratory tract infections. Although there is experimental evidence that gases and vapors can be adsorbed onto

carbonaceous particles, enhancing the toxicity of these particles when deposited in the lung, there is no evidence for an increased health risk from such interactions with DPM under urban atmospheric conditions. Likewise, there is no experimental evidence in laboratory animals that the youth or preexisting emphysema of an exposed individual enhances the risk of exposure to diesel exhaust.

5.4. COMPARATIVE RESPONSIVENESS AMONG SPECIES TO THE HISTOPATHOLOGIC EFFECTS OF DIESEL EXHAUST

There is some evidence indicating that species may differ in pulmonary responses to diesel exhaust. Mauderly (1994) compared the pulmonary histopathology of rats and mice after 18 mo of exposure to diesel exhaust. There was less aggregation of macrophages in rats. Diffuse septal thickening was noted in the mice, but there were few inflammatory cells, no focal fibrosis, little epithelial hyperplasia, and no epithelial metaplasia, as was observed in rats. Heinrich et al. (1986a) reported that wet lung weight of hamsters increased only 1.8-fold following chronic exposure to diesel exhaust, compared with an increase of 3.4-fold in rats. Smaller increases in neutrophils, lactic acid dehydrogenase, collagen, and protein supported the conclusion of a lesser inflammatory response in Syrian hamsters. The histopathologic changes in the lungs of Chinese hamsters after 6 mo exposure to diesel exhaust, on the other hand, was similar to that of rats (Pepelko and Peirano, 1983). Guinea pigs respond to chronic diesel exhaust exposure with a well-defined epithelial proliferation, but it is based on an eosinophilic response in contrast to the neutrophil-based responses in other species. Epithelial hyperplasia and metaplasia were quite striking in the terminal and respiratory bronchioles of cats exposed for 27 mo to diesel exhaust (Plopper et al., 1983). This study is of particular interest because the terminal airways of cats are more similar to those of humans than rodent species are. It should be noted, however, that exposure concentrations were very high (12 mg/m^3) for most of the period. Lewis et al. (1989) exposed rats and cynomolgus monkeys 8 h per day, 5 days per week for 2 years to diesel exhaust at a particle concentration of 2 mg/m^3. Unfortunately, this exposure rate was sufficiently low that few effects were noted in either species other than focal accumulations of particles, primarily in the alveolar macrophages, interstitium, and lymphoid tissue. It is apparent that species do vary in their pulmonary responses to diesel exhaust exposure, despite the difficulty in making direct comparisons because of differences in exposure regimes, lifespans, and pulmonary anatomy. Most species do respond, however, suggesting that humans are likely to be susceptible to induction of pulmonary pathology during chronic exposure to DE at some level.

5.5. DOSE-RATE AND PARTICULATE CAUSATIVE ISSUES

The purpose of animal toxicological experimentation is to elucidate mechanisms of action and identify the hazards and dose-response effects posed by a chemical substance or complex mixture and to extrapolate these effects to humans for subsequent health assessments. The cardinal principle in such a process is that the intensity and character of the toxic action are a function of the dose of the toxic agent(s) that reaches the critical site of action. The considerable body of evidence reviewed clearly denotes that major noncancerous health hazards may be presented to the lung following the inhalation of diesel exhaust. Based on pulmonary function and histopathological and histochemical effects, a determination can be made concerning which dose/exposure rates of diesel exhaust (expressed in terms of the DPM concentration) result in injury to the lung and which appear to elicit no effect. The inhalation of poorly soluble particles, such as those found in diesel exhaust, increases the pulmonary particulate burden. When the dosing rate exceeds the ability of the pulmonary defense mechanisms to achieve a steady-state lung burden of particles, there is a slowing of clearance and the progressive retention of particles in the lung that can ultimately approach a complete cessation of lung clearance (Morrow, 1988). This phenomenon, which is reviewed in Section 3.4, has practical significance both for the interpretation of experimental inhalation data and for the prevention of disease in humans exposed to airborne particles.

The data for exposure intensities that cause adverse pulmonary effects demonstrate that they are less than the exposure intensities reported to be necessary to induce lung tumors. Using the most widely studied laboratory animal species and the one reported to be the most sensitive to tumor induction, the laboratory rat, the no-adverse-effect exposure intensity for adverse pulmonary effects was 56 $mg \cdot h \cdot m^{-3}$/week (Brightwell et al., 1986). The lowest-observed-effect level for adverse pulmonary effects (noncancer) in rats was 70 $mg \cdot h \cdot m^{-3}$/week (Lewis et al., 1989), and for pulmonary tumors, 122.5 $mg \cdot h \cdot m^{-3}$/week (Mauderly et al., 1987a). The results clearly show that noncancerous pulmonary effects are produced at lower exposure intensities than are pulmonary tumors. Such data support the position that inflammatory and proliferative changes in the lung may play a key role in the etiology of pulmonary tumors in exposed rats (Mauderly et al., 1990b).

Adults who have a preexisting condition that may predispose their lungs to increased particle retention (e.g., smoking or high particulate burdens from nondiesel sources), inflammation (e.g., repeated respiratory infections), epithelial proliferation (e.g., chronic bronchitis), and fibrosis (e.g., silica exposure), as well as infants and children, because of their developing pulmonary and immunologic systems, may have a greater susceptibility to the toxic actions of diesel exhaust. It should be noted that both the developing lung and a model of a preexisting disease state have been studied with regard to their effect on the lungs' response to diesel exhaust (Mauderly et al., 1990a, 1987b). Mauderly et al. (1987b) showed that diesel did not affect the

developing lung more severely than the adult rat lung, and in fact, that clearance was faster in the younger lung. Mauderly et al. (1990a) compared the pulmonary response to inhalation of diesel exhaust in rats with elastase-induced emphysema with normal rats. They found that respiratory tract effects were not more severe in emphysematous rats and that the lung burden of particles was less in the compromised rat. These studies provide limited evidence that some factors that are often considered to result in a wider distribution of sensitivity among members of the population may not have this effect with diesel exposure. However, these studies have no counterpart in human studies and extrapolation to humans remains uncertain.

There is also the issue of whether the noncancerous health effects related to exposure to diesel exhaust are caused by the carbonaceous core of the particle or substances adsorbed onto the core, or both.

Current understanding, derived primarily from studies in rats, suggests that much of the toxicity resulting from the inhalation of diesel exhaust relates to the carbonaceous core of the particles. Several studies on inhaled aerosols demonstrate that lung reactions characterized by an appearance of particle-laden AMs and their infiltration into the alveolar ducts, adjoining alveoli, and tracheobronchial lymph nodes; hyperplasia of Type II cells; and the impairment of pulmonary clearance mechanisms are not limited to exposure to diesel particles. Such responses have also been observed in rats following the inhalation of coal dust (Lewis et al., 1989; Karagianes et al., 1981), titanium dioxide (Heinrich et al., 1995; Lee et al., 1985), CB (Nikula et al., 1995; Heinrich et al., 1995), titanium tetrachloride hydrolysis products (Lee et al., 1986), quartz (Klosterkötter and Bünemann, 1961), volcanic ash (Wehner et al., 1986), amosite (Bolton et al., 1983), and manmade mineral fibers (Lee et al., 1988) among others. In more recent studies, animals have been exposed to CB that is similar to the carbon core of the diesel exhaust particle. Nikula et al. (1995) exposed rats for 24 mo to CB or diesel exhaust at target exposure concentrations of 2.5 and 6 mg/m^3 (exposure rates of 200 or 520 mg·h·m^3/week). Both concentrations induced AM accumulation, epithelial proliferation, inflammation, and fibrosis. They observed essentially no difference in potency of nonneoplastic or in tumor responses based on a regression analysis.

Dungworth et al. (1994) reported moderate to severe inflammation characterized by multifocal bronchoalveolar hyperplasia, alveolar histiocytosis, and focal segmental fibrosis in rats exposed to CB for up to 20 mo at exposure rates of 510 to 540 mg·h·m^3/week. The observed lung pathology reflects notable dose-response relationships and usually evolves in a similar manner. With increasing dose, there is an increased accumulation and aggregation of particle-laden AMs, Type II cell hyperplasia, a foamy (degenerative) macrophage response, alveolar proteinosis, alveolar bronchiolization, cholesterol granulomas, and often squamous cell carcinomas and bronchioalveolar adenomas derived from metaplastic squamous cells in the areas of alveolar bronchiolization.

Heinrich et al. (1995) compared effects of diesel exposure in rats and mice with exposure to titanium dioxide or carbon black. Exposures to TiO_2 and carbon black were adjusted during the exposure to result in a similar lung burden for the three types of particles. At similar lung burdens in the rat, DPM, TiO_2, and CB had nearly identical effects on lung weights and on the incidence of lesions, both noncancer and cancer. Also, a similar effect on clearance of a labeled test aerosol was measured for the different particles. A comparison of the effect of DPM, TiO_2, and carbon black exposures in mice also showed a similar effect on lung weight, but noncancer effects were not reported and no significant increase in tumors was observed.

Murphy et al. (1998) compared the toxicological effects of DPM with three other particles chosen for their differing morphology and surface chemistry. One mg each of well-characterized crystalline quartz, amorphous silica, CB, and DPM was administered to laboratory rats by a single intratracheal instillation. The laboratory rats were sacrificed at 48 h, and 1, 6, and 12 weeks after instillation. Crystalline quartz produced significant increases in lung permeability, persistent surface inflammation, progressive increases in pulmonary surfactant and activities of epithelial marker enzymes up to 12 wk after primary exposure. Amorphous silica did not cause progressive effects but did produce initial epithelial damage with permeability changes that regressed with time after exposure. By contrast, CB had little if any effect on lung permeability, epithelial markers, or inflammation. Similarly, DPM produced only minimal changes, although the individual particles were smaller and differed in surface chemistry from CB. The authors concluded that DPM is less damaging to the respiratory epithelium than is silicon dioxide, and that the surface chemistry of the particle is more important than ultrafine size in explaining biological activity.

These experiments provide strong support for the idea that diesel exhaust toxicity results from a mechanism that is analogous to that of other relatively inert particles in the lung. This qualitative similarity exists along with some apparent quantitative differences in the potency of various particles for producing effects on the lung or on particle clearance.

The exact relationship between toxicity and particle size within the ultrafine particle mode, including DPM (BéruBé et al., 1999), remains unresolved. Studies reviewed in the PM CD (U.S. Environmental Protection Agency, 1996) suggest a greater inherent potential toxicity of inhaled ultrafine particles. Exposure to ultrafine particles may increase the release of proinflammatory mediators that could be involved in lung disease. For example, Driscoll and Maurer (1991) compared the effects of fine (0.3 µm) and ultrafine (0.02 µm) TiO_2 particles instilled into the lungs of laboratory rats. Although both size modes caused an increase in the numbers of AMs and PMNs in the lungs, and release of TNF and fibronectin by AMs, the responses were greater and more persistent with the ultrafine particles. While fine particle exposure resulted in a minimally increased prominence of particle-laden macrophages associated with alveolar ducts, ultrafine

particle exposure produced a somewhat greater prominence of macrophages, some necrosis of macrophages, and slight interstitial inflammation of the alveolar duct region. Moreover, collagen increased only with exposure to ultrafine particles.

Oberdörster et al. (1992) compared the effects of fine (0.25 μm) and ultrafine (0.02 μm) TiO_2 particles instilled into the lungs of laboratory rats on various indicators of inflammation. Instillation of ultrafine particles increased the number of total cells recovered by lavage, decreased the percentage of AMs, and increased the percentage of PMNs and protein. Instillation with fine particles did not cause statistically significant effects. Thus, the ultrafine particles had greater pulmonary inflammatory potency than did larger sizes of this material. The investigators attributed the enhanced toxicity to greater interaction of the ultrafine particles with their large surface area, with alveolar and interstitial macrophages, which resulted in enhanced release of inflammatory mediators. They suggested that ultrafine particles of low in vitro solubility appear to enter the interstitium more readily than do larger sizes of the same material, which accounted for the increased contact with macrophages in this compartment of the lung. Driscoll and Maurer (1991) noted that the pulmonary retention of ultrafine TiO_2 particles instilled into rat lungs was greater than for the same mass of fine-mode TiO_2 particles. Thus, the available evidence tends to suggest a potentially greater toxicity for inhaled ultrafine particles.

Particle size, volume, surface area, and composition may be the critical elements in the overload phenomenon following exposure to particles, which could explain those quantitative differences. The overloaded AMs secrete a variety of cytokines, oxidants, and proteolytic enzymes that are responsible for inducing particle aggregation and damaging adjacent epithelial tissue (Oberdörster, 1994). For a more detailed discussion of mechanism, see Chapter 3.

The principal noncancerous health hazard to humans posed by exposure to diesel exhaust is a structural or functional injury to the lung, on the basis of the laboratory animal data. Such effects are demonstrable at dose rates or cumulative doses of DPM lower than those reported to be necessary to induce lung tumors. An emerging human health issue concerning short-term exposure to ambient DE/DPM is the potential for allergenic responses in several studies. Heightened allergenic responses including increased cytokine production as well as increased numbers of inflammatory cells have been detected in nasal lavage from humans exposed to inhaled or instilled DE/DPM. In individuals already allergic to ragweed, exposure to DE/DPM with the allergen was observed to result in an enhanced allergenic response, particularly IgE production. Current knowledge indicates that the carbonaceous core of diesel particles is the major causative factor in the injury to the lung and that other factors such as the cytotoxicity of adsorbed substances on the particles also may play a role. The lung injury appears to be mediated through effects on pulmonary AMs. Because noncancerous pulmonary effects occur at lower doses than tumor induction does in the rat, and because these effects may be cofactors in the etiology of

diesel exhaust-induced tumors, noncancerous pulmonary effects must be considered in the total evaluation of diesel exhaust, notably the particulate component.

5.6. SUMMARY AND DISCUSSION

5.6.1. Effects of Diesel Exhaust on Humans

The most readily identified acute noncancer health effect of diesel exhaust on humans is its ability to elicit subjective complaints of eye, throat, and bronchial irritation and neurophysiological symptoms such as headache, lightheadedness, nausea, vomiting, and numbness and tingling of the extremities. Studies of the perception and offensiveness of the odor of diesel exhaust and a human volunteer study in an exposure chamber have demonstrated that the time of onset of the human subjective symptoms is inversely related to increasing concentrations of diesel exhaust and the severity is directly related to increasing concentrations of diesel exhaust. In one study in which a diesel engine was operated under varying load conditions, a dilution factor of 140 to 475 was needed to reduce the exhaust level to an odor-detection threshold level.

A public health issue is whether short-term exposure to diesel exhaust might result in an acute decrement in ventilatory function and whether the frequent repetition of such acute respiratory effects could result in chronic lung function impairment. One convenient means of studying acute decrements in ventilatory function is to monitor differences in pulmonary function in occupationally exposed workers at the beginning and end of a workshift. In studies of underground miners, bus garage workers, dockworkers, and locomotive repairmen, increases in respiratory symptoms (cough, phlegm, and dyspnea) and decreases in lung function (FVC, FEV_1, PEFR, and FEF_{25-75}) over the course of a workshift were generally found to be minimal and not statistically significant. In a study of acute respiratory responses in diesel bus garage workers, there was an increased reporting of cough, labored breathing, chest tightness, and wheezing, but no reductions in pulmonary function were associated with exposure to diesel exhaust. Pulmonary function was affected in stevedores over a workshift exposure to diesel exhaust but normalized after a few days without exposure to diesel exhaust fumes. In a third study, there was a trend toward greater ventilatory function changes during a workshift among coal miners, but the decrements were similar in miners exposed and not exposed to diesel exhaust.

Smokers appeared to demonstrate larger workshift respiratory function decrements and increased incidents of respiratory symptoms. Acute sensory and respiratory symptoms were earlier and more sensitive indicators of potential health risks from diesel exposure than were decrements in pulmonary function. Studies on the acute health effects of exposure to diesel exhaust in humans, experimental and epidemiologic, have failed to demonstrate a consistent pattern of adverse effects on respiratory morbidity; the majority of studies offer, at best, equivocal evidence for an exposure-response relationship. The environmental contaminants have frequently

been below permissible workplace exposure limits; in those few cases where health effects have been reported, the authors have failed to identify conclusively the individual or collective causative agents in the diesel exhaust.

Chronic effects of diesel exhaust exposure have been evaluated in epidemiologic studies of occupationally exposed workers (metal and nonmetal miners, railroad yard workers, stevedores, and bus garage mechanics). Most of the epidemiologic data indicate an absence of an excess risk of chronic respiratory disease associated with exposure to diesel exhaust. In a few studies, a higher prevalence of respiratory symptoms, primarily cough, phlegm, or chronic bronchitis, was observed among the exposed. These increased symptoms, however, were usually not accompanied by significant changes in pulmonary function. Reductions in FEV_1 and FVC and, to a lesser extent, FEF_{50} and FEF_{75}, also have been reported. Two studies detected statistically significant decrements in baseline pulmonary function consistent with obstructive airway disease. One study of stevedores had a limited sample size of 17 exposed and 11 controls. The second study in coal miners showed that both underground and surface workers at diesel-use mines had somewhat lower pulmonary performance than their matched controls. The proportion of workers in or at diesel-use mines, however, showed equivalent evidence of obstructive airway disease, and for this reason the authors of the second paper felt that factors other than diesel exposure might have been responsible. A doubling of minor restrictive airway disease was also observed in workers in or at diesel-use mines. These two studies, coupled with other reported nonsignificant trends in respiratory flow-volume measurements, suggest that exposure to diesel exhaust may impair pulmonary function among occupational populations. Epidemiologic studies of the effects of diesel exhaust on organ systems other than the pulmonary system are scant. Whereas a preliminary study of the association of cardiovascular mortality and exposure to diesel exhaust found a fourfold higher risk ratio, a more comprehensive epidemiologic study by the same investigators found no significant difference between the observed and expected number of deaths caused by cardiovascular disease.

Caution is warranted in the interpretation of results from the epidemiologic studies that have addressed noncarcinogenic health effects from exposure to diesel exhaust. These investigations suffer from myriad methodological problems, including (1) incomplete information on the extent of exposure to diesel exhaust, necessitating in some studies estimations of exposures from job titles and resultant misclassification; (2) the presence of confounding variables such as smoking or occupational exposures to other toxic substances (e.g., mine dusts); and (3) the short duration and low intensity of exposures. These limitations restrict drawing definitive conclusions as to the cause of any noncarcinogenic diesel exhaust effect, observed or reported.

It is also apparent that at some level of exposure DE as measured by DPM has the potential to induce systemic and pulmonary inflammatory responses in healthy humans and in stimulating allergen-induced allergic airway disease in sensitive humans.

5.6.2. Effects of Diesel Exhaust on Laboratory Animals

Laboratory animal studies of the toxic effects of diesel exhaust have involved acute, subchronic, and chronic exposure regimens. In acute exposure studies, toxic effects appear to have been associated primarily with high concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. In short- and long-term studies, toxic effects have been associated with exposure to the complex exhaust mixture. Effects of diesel exhaust in various animal species are summarized in Tables 5-2 to 5-15. In short-term studies, health effects are not readily apparent, and when found, are mild and result from concentrations of about 6 mg/m^3 DPM and durations of exposure approximating 20 h/day. There is ample evidence, however, that short-term exposures at lower levels of diesel exhaust affect the lung, as indicated by an accumulation of DPM, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and the thickening of alveolar walls adjacent to AM aggregation. Little evidence exists, however, from short-term studies that exposure to diesel exhaust impairs lung function. Chronic exposures cause lung pathology that results in altered pulmonary function and increased DPM retention in the lung. Exposures to diesel exhaust have also been associated with increased susceptibility to respiratory tract infection, neurological or behavioral changes, an increase in banded neutrophils, and morphological alterations in the liver.

5.6.2.1. *Effects on Survival and Growth*

The data presented in Table 5-3 show limited effects on survival in mice and rats and some evidence of reduced body weight in rats following chronic exposures to concentrations of 1.5 mg/m^3 DPM or higher and exposure durations of 16 to 20 h/day, 5 days/week for 104 to 130 weeks. Increased lung weights and lung to body-weight ratios in rats, mice, and hamsters; an increased heart to body weight ratio in rats; and decreased lung and kidney weights in cats have been reported following chronic exposure to diesel exhaust. No evidence was found of an effect of diesel exhaust on other body organs (Table 5-4). The lowest-observed-effect level in rats approximated 1 to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 104 weeks.

5.6.2.2. *Effects on Pulmonary Function*

Pulmonary function impairment has been reported in rats, hamsters, cats, and monkeys exposed to diesel exhaust and included lung mechanical properties (compliance and resistance), diffusing capacity, lung volumes, and ventilatory performance (Table 5-5). The effects generally

appeared only after prolonged exposures. The lowest exposure levels (expressed in terms of DPM concentrations) that resulted in impairment of pulmonary function occurred at 2 mg/m^3 in cynomolgus monkeys (the only level tested), 1.5 and 3.5 mg/m^3 in rats, 4.24 and 6 mg/m^3 in hamsters, and 11.7 mg/m^3 in cats. Exposures in monkeys, cats, and rats (3.5 mg/m^3) were for 7 to 8 h/day, 5 days/week for 104 to 130 weeks. While this duration is considered to constitute a lifetime study in rodents, it is a small part of the lifetime of a monkey or cat. Exposures in hamsters and rats (1.5 mg/m^3) varied in hours per day (8 to 20) and weeks of exposure (26 to 130). In all species but the monkey, the testing results were consistent with restrictive lung disease; alteration in expiratory flow rates indicated that 1.5 mg/m^3 DPM was a LOAEL for a chronic exposure (Gross, 1981). Monkeys demonstrated evidence of obstructive airway disease. The nature of the pulmonary impairment is dependent on the dose of toxicants delivered to and retained in the lung, the site of deposition and effective clearance or repair, and the anatomy and physiology of the affected species; these variables appear to be factors in the disparity of the airway disease in monkey versus the other species tested.

5.6.2.3. *Histopathological and Histochemical Effects*

Histological studies have demonstrated that chronic exposure to diesel exhaust can result in effects on respiratory tract tissue (Table 5-6). Typical findings include alveolar histiocytosis, AM aggregation, tissue inflammation, increase in PMNs, hyperplasia of bronchiolar and alveolar Type II cells, thickened alveolar septa, edema, fibrosis, and emphysema. Lesions in the trachea and bronchi were observed in some studies. Associated with these histopathological findings were various histochemical changes in the lung, including increases in lung DNA, total protein, alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase; increased synthesis of collagen; and release of inflammatory mediators such as leukotriene LTB and prostaglandin $PGF_{2\alpha}$. Although the overall laboratory evidence is that prolonged exposure to DPM results in histopathological and histochemical changes in the lungs of exposed animals, some studies have also demonstrated that there may be a threshold of exposure to DPM below which pathologic changes do not occur. These no-observed-adverse-effect levels for histopathological effects were reported to be 2 mg/m^3 for cynomolgus monkeys (the only concentration tested), 0.11 to 0.35 mg/m^3 for rats, and 0.25 mg/m^3 DPM for guinea pigs exposed for 7 to 20 h/day, 5 to 5.5 days/week for 104 to 130 weeks.

5.6.2.4. *Effects on Airway Clearance*

The pathological effects of DPM appear to be strongly dependent on the relative rates of pulmonary deposition and clearance (Table 5-7). Clearance of particles from the alveolar region of the lungs is a multiphasic process involving phagocytosis by AMs. Chronic exposure to DPM

concentrations of about 1 mg/m^3 or above, under varying exposure durations, causes pulmonary clearance to be reduced, with concomitant focal aggregations of particle-laden AMs, particularly in the peribronchiolar and alveolar regions, as well as in the hilar and mediastinal lymph nodes. The exposure concentration at which focal aggregates of particle-laden AMs occur may vary from species to species, depending on rate of uptake and pulmonary deposition, pulmonary clearance rates, the relative size of the AM population per unit of lung tissue, the rate of recruitment of AMs and leukocytes, and the relative efficiencies for removal of particles by the mucociliary and lymphatic transport system. The principal means by which PM clearance is reduced is through a decrease in the function of pulmonary AMs. Impairment of particle clearance seems to be nonspecific and applies primarily to dusts that are persistently retained in the lungs. Lung dust levels of approximately 0.1 to 1 mg/g lung tissue appear to produce this effect in the Fischer 344 rat (Health Effects Institute, 1995). Morrow (1988) suggested that the inability of particle-laden AMs to translocate to the mucociliary escalator is correlated to an average composite particle volume per AM in the lung. When this particle volume exceeds approximately 60 μm^3 per AM in the Fischer 344 rat, impairment of clearance appears to be initiated. When the particulate volume exceeds approximately 600 μm^3 per cell, evidence suggests that AM-mediated particulate clearance virtually ceases, agglomerated particle-laden macrophages remain in the alveolar region, and increasingly nonphagocytized dust particles translocate to the pulmonary interstitium. Data for other laboratory animal species and humans are, unfortunately, limited.

5.6.2.5. *Neurological and Behavioral Effects*

Behavioral effects have been observed in rats exposed to diesel exhaust from birth to 28 days of age (Table 5-14). Exposure caused a decreased level of spontaneous locomotor activity and a detrimental effect on learning in adulthood. In agreement with the behavioral changes was physiological evidence for delayed neuronal maturation. Exposures were to 6 mg/m^3 DPM for 8 h/day, 7 days/week from birth to about 7, 14, 21, or 28 days of age.

5.6.2.6. *Effects on Immunity and Allergenicity*

Several laboratory animal studies have indicated that exposure to DPM can reduce an animal's resistance to respiratory infection. This effect, which can occur even after only 2 or 6 h of exposure to DE containing 5 to 8 mg/m^3 DPM, does not appear to be caused by direct impairment of the lymphoid or splenic immune systems; however, in one study of influenza virus infection, interferon levels and hemaglutinin antibody levels were adversely affected in the exposed mice.

As with humans, there are animal data suggesting that DPM is a possible factor in the increasing incidence of allergic hypersensitivity. The effects have been demonstrated primarily in

acute human and laboratory animal studies and appear to be associated with both the nonextractable carbon core and the organic fraction of DPM. It also appears that synergies with DPM may increase the efficacy of known airborne allergens. Both animal and human cell culture studies indicate that DPM also has the potential to act as an adjuvant.

5.6.2.7. *Other Noncancer Effects*

Essentially no effects (based on the weight of evidence of a number of studies) were noted for reproductive and teratogenic effects in mice, rats, rabbits, and monkeys; clinical chemistry and hematology in the rat, cat, hamster, and monkeys; and enzyme induction in the rat and mouse (Tables 5-11 through 5-13 and 5-15).

5.6.3. Comparison of Filtered and Unfiltered Diesel Exhaust

The comparison of the toxic responses in laboratory animals exposed to whole diesel exhaust or filtered exhaust containing no particles demonstrates across laboratories that diesel particles are the principal etiologic agent of noncancerous health effects in laboratory animals exposed to diesel exhaust (Table 5-16). Whether the particles act additively or synergistically with the gases cannot be determined from the designs of the studies. Under equivalent exposure regimens, hamsters have lower levels of retained DPM in their lungs than rats and mice do and consequently less pulmonary function impairment and pulmonary pathology. These differences may result from a lower intake rate of DPM, lower deposition rate and/or more rapid clearance rate, or lung tissue that is less susceptible to the cytotoxicity of DPM. Observations of a decreased respiration in hamsters when exposed by inhalation favor lower intake and deposition rates.

5.6.4. Interactive Effects of Diesel Exhaust

There is no direct evidence that diesel exhaust interacts with other substances in an exposure environment, other than an impaired resistance to respiratory tract infections. Young animals were not more susceptible. In several ways, animals with laboratory-induced emphysema were more resistant. There is experimental evidence that both inorganic and organic compounds can be adsorbed onto carbonaceous particles. When such substances become affiliated with particles, these substances can be carried deeper into the lungs where they might have a more direct and potent effect on epithelial cells or on AM ingesting the particles. Few specific studies to test interactive effects of diesel exhaust with atmospheric contaminants, other than coal dust, have been conducted. Coal dust and DPM had an additive effect only.

5.6.5. Conclusions

Conclusions concerning the principal human hazard from exposure to diesel exhaust are as follows:

- Some occupational studies of acute exposure to diesel exhaust during work shifts suggest that increased acute sensory and respiratory symptoms (cough, phlegm, chest tightness, wheezing) are more sensitive indicators of possible health risks from exposure to diesel exhaust than pulmonary function decrements (which were consistently found not to be significantly associated with diesel exhaust exposure).
- Allergenic effects also have been demonstrated under short-term exposure scenarios to either diesel exhaust or DPM. The evidence indicates that the immunological changes appear to be due to the DPM component of diesel exhaust and that the immunological changes are caused by both the non extractable carbon core and the adsorbed organic fraction of the diesel particle. The toxicological significance of these effects has yet to be resolved.
- Noncancer effects in humans from long-term chronic exposure to DPM are not evident. Noncancer effects from long-term exposure to DPM of several laboratory animal species, conducted to assess the pathophysiologic effects of DPM in humans showed pulmonary histopathology and chronic inflammation.

Although the mode of action of DE is not clearly evident for any of the effects documented in this chapter, the respiratory tract effects observed under acute scenarios are suggestive of an irritant mechanism, while lung effects observed in chronic scenarios indicate an underlying inflammatory response. Current knowledge indicates that the carbonaceous core of the diesel particle is the causative agent of the lung effects, with the extent of the injury being mediated at least in part by a progressive impairment of AMs. It is noted that lung effects occur in response to DE exposure in several species and occur in rats at doses lower than those inducing particle overload and a tumorigenic response (see above); it follows that lung effects such as inflammation and fibrosis are relevant in the development of risk assessments for DE.

Table 5-1. Human studies of exposure to diesel exhaust

Study	Description	Findings
	Acute exposures	
Kahn et al. (1988)	13 cases of acute exposure, Utah and Colorado coal miners.	Acute reversible sensory irritation, headache, nervous system effects, bronchoconstriction were reported at unknown exposures.
El Batawi and Noweir (1966)	161 workers, two diesel bus garages.	Eye irritation (42%), headache (37%), dizziness (30%), throat irritation (19%), and cough and phlegm (11%) were reported in this order of incidence by workers exposed in the service and repair of diesel-powered buses.
Battigelli (1965)	Six subjects, eye exposure chamber, three dilutions.	Time to onset was inversely related and severity of eye irritation was associated with the level of exposure to diesel exhaust.
Katz et al. (1960)	14 persons monitoring diesel exhaust in a train tunnel.	Three occasions of minor eye and throat irritation; no correlation established with concentrations of diesel exhaust components.
Hare and Springer (1971) Hare et al. (1974)	Volunteer panelists who evaluated general public's response to odor of diesel exhaust.	Slight odor intensity, 90% perceived, 60% objected; slight to moderate odor intensity, 95% perceived, 75% objected; moderate odor intensity, 100% perceived, almost 95% objected.
Linnell and Scott (1962)	Odor panel under highly controlled conditions determined odor threshold for diesel exhaust.	In six panelists, the volume of air required to dilute raw diesel exhaust to an odor threshold ranged from a factor of 140 to 475.
Rudell et al. (1990, 1994)	Eight healthy nonsmoking subjects exposed for 60 min in chamber to diesel exhaust (3.7 ppm NO, 1.5 ppm NO_2, 27 ppm CO, 0.5 mg/m^3 formaldehyde, particles (4.3 $\times 10^6$/cm^3). Exercise, 10 of each 20 min (75 W).	Odor, eye and nasal irritation in 5/8 subjects. BAL findings: small decrease in mast cells, lymphocyte subsets and macrophage phagocytosis; small increase in PMNs.
Rudell et al. (1996)	Volunteers exposed to diesel exhaust for 1 h while doing light work. Exposure concentrations uncertain.	Unpleasant smell along with irritation of eyes and nose reported. Airway resistance increased. Reduction of particle concentration by trapping did not affect results.
Battigelli (1965)	13 volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 h.	No significant effects on pulmonary resistance were observed as measured by plethysmography.
Wade and Newman (1993)	Three railroad workers acutely exposed to diesel exhaust.	The workers developed symptoms of asthma.

Diaz-Sanchez et al. (1994)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	Enhancement of IgE production reported due to a dramatic increase in IgE-secreting cells.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Takenaka et al. (1995)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	DPM extracts enhanced interleukin-4 plus monoclonal antibody-stimulated IgE production as much as 360%, suggesting an enhancement of ongoing IgE production rather than inducing germline transcription or isotype switching.
Diaz-Sanchez et al. (1996)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	A broad increase in cytokine expression predicted to contribute to enhanced local IgE production.
Diaz-Sanchez et al. (1997)	Ragweed-sensitive volunteers challenged by a nasal spray of 0.30 mg DPM alone or in combination with ragweed allergen.	Ragweed allergen plus DPM-stimulated ragweed-specific IgE to a much greater degree than ragweed alone, suggesting DPM may be a key feature in stimulating allergen-induced respiratory allergic disease.
Salvi et al. (1999)	Volunteers exposed to diluted diesel exhaust (DPM 300 $\mu g/m^3$) for 1 h with intermittent exercise.	• No changes in pulmonary function, but significant increases in neutrophils, B lymphocytes, histamine, and fibronectin in airway lavage fluid. • Bronchial biopsies 6 h after exposure showed significant increase in neutrophils, mast cells, CD4+ and CD8+ T lymphocytes; upregulation of ICAM-1 and VCAM-1; increases in the number of LFA-1+ in bronchial tissue. • Significant increases in neutrophils and platelets observed in peripheral blood.
Salvi et al. (2000)	Volunteers exposed to diluted diesel exhaust (DPM 300 $\mu g/m^3$) for 1 h.	• DPM enhanced gene transcription of IL-8 in bronchial tissue and bronchial wash cells • Increased expression of growth-regulated oncogene-α and IL-8 in bronchial epithelium; trend towards increased IL-5 mRNA gene transcripts.
	Studies of cross-shift changes	
Reger (1979)	Five or more VC maneuvers by each of 60 coal miners exposed to diesel exhaust at the beginning and end of a workshift.	FEV_1, FVC, and PEFR were similar between diesel and non-diesel-exposed miners. Smokers had an increased number of decrements over shift than nonsmokers.

Ames et al. (1982)	Pulmonary function of 60 diesel-exposed compared with 90 non-diesel-exposed coal miners over workshift.	Significant workshift decrements occurred in miners in both groups who smoked; no significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Jörgensen and Svensson (1970)	240 iron ore miners matched for diesel exposure, smoking, and age were given bronchitis questionnaires and spirometry pre- and postworkshift.	Among underground (surrogate for diesel exposure) miners, smokers, and older age groups, frequency of bronchitis was higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age.
Gamble et al. (1979)	200 salt miners performed before- and after-workshift spirometry. Personal environmental NO_2 and inhalable particle samples were collected.	Smokers had greater but not significant reductions in spirometry than ex- or nonsmokers. NO_2 but not particulate levels significantly decreased FEV1, FEF_{25}, FEF_{50}, and FEF_{75} over the workshift.
Gamble et al. (1987a)	232 workers in 4 diesel bus garages administered acute respiratory questionnaire and before and after workshift spirometry. Compared to lead/acid battery workers previously found to be unaffected by their exposures.	Prevalence of burning eyes, headache, difficult or labored breathing, nausea, and wheeze were higher in diesel bus workers than in comparison population.
Ulfvarson et al. (1987)	Workshift changes in pulmonary function were evaluated in crews of roll-on/ roll-off ships and car ferries and bus garage staff. Pulmonary function was evaluated in six volunteers exposed to diluted diesel exhaust, 2.1 ppm NO_2, and 0.6 mg/m^3 particulate matter.	Pulmonary function was affected during a workshift exposure to diesel exhaust, but it normalized after a few days with no exposure. Decrements were greater with increasing intervals between exposures. No effect on pulmonary function was observed in the experimental exposure study.
	Cross-sectional and longitudinal studies	
Battigelli et al. (1964)	210 locomotive repairmen exposed to diesel exhaust for an average of 9.6 years in railroad engine houses were compared with 154 railroad yard workers of comparable job status but no exposure to diesel exhaust.	No significant differences in VC, FEV_1, peak flow, nitrogen washout, or diffusion capacity or in the prevalence of dyspnea, cough, or sputum were found between the diesel exhaust-exposed and nonexposed groups.

Gamble et al. (1987b)	283 male diesel bus garage workers from four garages in two cities were examined for impaired pulmonary function (FVC, FEV_1, and flow rates). Study population with a mean tenure of 9 ± 10 years S.D. was compared to a nonexposed blue-collar population.	Analyses within the study population showed no association of respiratory symptoms with tenure. Reduced FEV_1 and FEF_{50} (but not FEF_{75}) were associated with increasing tenure. The study population had a higher incidence of cough, phlegm, and wheezing unrelated to tenure. Pulmonary function was not affected in the total cohort of diesel-exposed but was reduced with 10 or more years of tenure.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Purdham et al. (1987)	Respiratory symptoms and pulmonary function were evaluated in 17 stevedores exposed to both diesel and gasoline exhausts in car ferry operations; control group was 11 on-site office workers.	No differences between the two groups for respiratory symptoms. Stevedores had lower baseline lung function consistent with an obstructive ventilatory defect compared with controls and those of Sydney, Nova Scotia, residents. Caution in interpretation is warranted because of small sample size. No significant changes in lung function over workshift or difference between two groups.
Reger et al. (1982)	Differences in respiratory symptoms and pulmonary function were assessed in 823 coal miners from 6 diesel-equipped mines compared to 823 matched coal miners not exposed to diesel exhaust.	Underground miners in diesel-use mines reported more symptoms of cough and phlegm and had lower pulmonary function. Similar trends were noted for surface workers at diesel-use mines. Pattern was consistent with small airway disease but factors other than exposure to diesel exhaust thought to be responsible.
Ames et al. (1984)	Changes in respiratory symptoms and function were measured during a 5-year period in 280 diesel-exposed and 838 nonexposed U.S. underground coal miners.	No decrements in pulmonary function or increased prevalence of respiratory symptoms were found attributable to diesel exhaust. In fact, 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in miners exposed to diesel exhaust.
Attfield (1978)	Respiratory symptoms and function were assessed in 2,659 miners from 21 underground metal mines (1,709 miners) and nonmetal mines (950 miners). Years of diesel usage in the mines were surrogate for exposure to diesel exhaust.	Questionnaire found an association between an increased prevalence of cough and aldehyde exposure; this finding was not substantiated by spirometry data. No adverse symptoms or pulmonary function decrements were related to exposure to NO_2, CO, CO_2, dust, or quartz.

Attfield et al. (1982)	Respiratory symptoms and function were assessed in 630 potash miners from 6 potash mines through a questionnaire, chest radiographs, and spirometry. A thorough assessment of the environment of each mine was made concurrently.	No obvious association indicative of diesel exposure was found between health indices, dust exposure, and pollutants. Higher prevalences of cough and phlegm but no differences in FVC and FEV_1 were found in these diesel-exposed potash workers when compared with predicted values from a logistic model based on blue-collar staff working in nondusty jobs.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Gamble et al. (1983)	Respiratory morbidity was assessed in 259 miners in 5 salt mines by respiratory symptoms, radiographic findings, and spirometry. Two mines used diesels extensively, two had limited use, and one used no diesels in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt-mine cohort.	After adjustment for age and smoking, salt miners showed no symptoms or increased prevalence of cough, phlegm, dyspnea, or air obstruction (FEV_1/FVC) compared with aboveground coal miners, potash workers, or blue-collar workers. FEV_1, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in comparison with all the comparison populations. No changes in pulmonary function were associated with years of exposure or cumulative exposure to inhalable particles or NO_2.
Gamble and Jones (1983)	Same as above. Salt miners were grouped into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exposure.	A statistically significant dose-related association of phlegm and diesel exposure was noted. Changes in pulmonary function showed no association with diesel tenure. Age- and smoking-adjusted rates of cough, phlegm, and dyspnea were 145%, 169%, and 93% of an external comparison population. Predicted pulmonary function indices showed small but significant reductions; there was no dose-response relationship.
Edling and Axelson (1984)	Pilot study of 129 bus company employees classified into 3 diesel-exhaust exposure categories: clerks (0), bus drivers (1), and bus garage workers.	The most heavily exposed group (bus garage workers) had a fourfold increase in risk of dying from cardiovascular disease, even after correction for smoking and allowing for 10 years of exposure and 14 years or more of induction latency time.
Edling et al. (1987)	Cohort of 694 male bus garage employees followed from 1951 through 1983 was evaluated for mortality from cardiovascular disease. Subcohorts categorized by levels of exposure were clerks (0), bus drivers (1), and bus garage employees (2).	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as subcohorts with different levels of exposure.

Table 5-2. Short-term effects of diesel exhaust on laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 10-13 weeks	1.5 0.19 μm MMD	2,100 to 2,730	6.9	0.49	—	Increase in lung wt; increase in thickness of alveolar walls; minimal species difference	Kaplan et al. (1982)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 19 weeks	0.21 1.0 4.4	140 665 2,926	— — —	— — —	— — —	No effects on lung function in rats (not done in mice); increase in PMNs and proteases and AM aggregation in both species	Mauderly et al. (1981)
Cat, Inbred, M	20 h/day 7 days/week 4 weeks	6.4	3,584	14.6	2.1	2.1	Few effects on lung function; focal pneumonitis or alveolitis	Pepelko et al. (1980a)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 4 weeks	6.4 6.8[a]	3,584 3,808	16.9 16.1[a]	2.49 2.76[a] (<0.01 ppm O_3)[a]	2.10 1.86[a]	Decreased body wt; arterial blood pH reduced; vital capacity, total lung capacities increased	Pepelko (1982a)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 4 weeks	6.8[a]	3,808	16.7	2.9 (<0.01 ppm O_3)[a]	1.9	Exposure started when animals were 4 days old; increase in pulmonary flow; bardycardia	Wiester et al. (1980)
Rat, F344, M	20 h/day 5.5 days/week 4 weeks	6.0 6.8 μm MMD	2,640	—	—	—	Macrophage aggregation; increase in PMNs; Type II cell proliferation; thickened alveolar walls	White and Garg (1981)
Guinea Pig, Hartley, M	30 min	1-2 mg DPM Intranasally	—	—	—	—	Augmented increases in nasal airway resistance and vascular permeability induced by a histamine aerosol	Kobayashi and Ito (1995)
Guinea Pig, Hartley, M	3 h	1 3.2	0.5 1.6	5.9 12.9	1.4 4.4	0.13 0.34	Similar results to those reported in the previous study using intranasal challenge	Kobayashi et al. (1997)

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Table 5-2. Short-term effects of diesel exhaust on laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3	7,056	17.4	2.3	2.1	Increase in relative lung wt. AM aggregation; hypertrophy of goblet cells; focal hyperplasia of alveolar epithelium	Wiester et al. (1980)
Mouse ICR, M	6 weeks	100 μg DPM intranasally	—	—	—	—	DPM aggravated ovalbumin-induced airway inflammation and provided evidence that DPM can enhance manifestations of allergic asthma	Takano et al. (1997)
Rat, Sprague-Dawley, M	24 h	5-100 $\mu g/10^6$ AM/mL of DPM	—	—	—	—	Unchanged, but not organic-free DPM enhanced production of proinflammatory cytokines	Yang et al. (1997)

[a]Irradiated exhaust.
PMN = Polymorphonuclear leukocyte.
AM = Alveolar macrophage.

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Table 5-3. Effects of chronic exposures to diesel exhaust on survival and growth of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F; Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MMD	7,280	11.5	1.5	0.8	No effects on growth or survival	Lewis et al. (1989)
Rat, F344, M; Guinea Pig, Hartley, M	20 h/day 5 days/week 106 weeks	0.25 0.75 1.5 0.19 μm MMD	2,650 7,950 15,900	2.7[a] 4.4[a] 7.1[a]	0.1[b] 0.27[b] 0.5[b]	— — —	Reduced body weight in rats at 1.5 mg/m^3	Schreck et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	No effect on growth	Vinegar et al. (1981a,b)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8.3 0.71 μm MMD	21,663	50.0	4.0–6.0	—	No effect on growth or mortality rates	Karagianes et al. (1981)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.25 μm MMD	1,592 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	No effect on growth or mortality rates	Mauderly et al. (1984, 1987a)
Rat, Wistar, F; Mouse, MMRI, F	19 h/day 5 days/week 104 weeks	4.24 0.35 μm MMD	41,891	12.5	1.5	1.1	Reduced body wts; increased mortality in mice	Heinrich et al. (1986a)
Rat, F344 M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Growth reduced at 2.2 and 6.6 mg/m^3	Brightwell et al. (1986)
Rat[c] F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11[d] 0.41[d] 1.08[d] 2.31[d] 3.72[e] 0.2–0.3 μm MMD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Concentration-dependent decrease in body weight; earlier deaths in females exposed to 3.72 mg/m^3, stabilized by 15 mo	Research Committee for HERP Studies (1988)
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/week 24 mo	0.84 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Reduced body weight in rats at 2.5 and 6.98 mg/m^3 and no effect in mice	Heinrich et al. (1995)

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Table 5-3. Effects of chronic exposures to diesel exhaust on survival and growth of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mice, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	Reduced body weight in NMRI mice but not in C57BL/6N mice	Heinrich et al. (1995)
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Reduced survival in 6.33 mg/m^3 after 300 days. Body weight significantly lower at 6.33 mg/m^3	Nikula et al. (1995)
Mouse, CD-1, M,F	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 μm MDD	1,274 12,740 25,844	3 17 30	0.1 0.3 0.7	— — —	No effect on growth or mortality rates	Mauderly et al. (1996)

[a]Estimated from graphically depicted mass concentration data.
[b]Estimated from graphically presented mass concentration data for NO_2 (assuming 90% NO and 10% NO_2).
[c]Data for tests with light-duty engine; similar results with heavy-duty engine.
[d]Light-duty engine.
[e]Heavy-duty engine.

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Table 5-4. Effects of chronic exposures to diesel exhaust on organ weights and organ-to-body-weight ratios

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 12-13 weeks	1.5 0.19 μm MMD	2,520-2,730	—	—	—	No effect on liver, kidney, spleen, or heart weights	Kaplan et al. (1982)
Rat, F344, M, F	7 h/day 5 days/week 52 weeks	2.0 0.23–0.36 μm MMD	3,640	12.7	1.6	0.83	No effects on weights of lungs, liver, heart, spleen, kidneys, and testes	Green et al. (1983)
Rat, F344, M	20 h/day 5.5 days/ week 36 weeks	0.25 1.5 0.19 μm MMD	990 5,940	— —	— —	— —	Increase in relative lung weight at 1.5 mg/m^3 only initially seen at 12 weeks	Misiorowski et al. (1980)
Rat, F344, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MMD	7,280	11.5	1.5	0.81	No effects on heart weights	Vallyathan et al. (1986)
Rat, F344; M Guinea Pig, Hartley, M	20 h/day 5.5 days/ week 78 weeks	0.25 0.75 1.5 0.19 μm MMD	2,145 6,435 12,870	— — —	— — —	— — —	No effects on heart mass	Penney et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	Increase in lung weight and lung/body weight ratio	Vinegar et al. (1981a,b)
Rat, Wistar, F; Hamster, Syrian, M, F Mouse, NMRI, F	19 h/day 5 days/week 120-140 weeks	4.24 0.35 μm MMD	48,336-56,392	12.5	1.5	1.1	Increase in rat, mouse, and hamster lung weight and dry weights	Heinrich et al. (1986a,b) Stöber (1986)
Rat, F344, M, F; Hamster, Syrian, M, F	16 h/day 5 days/week 104 weeks	0.7[a] 2.2[b] 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Increase in lung weight concentration related in rats; heart weight/body weight ratio greater at 6.6 mg/m^3	Brightwell et al. (1986)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[a] 12.0[b]	41,664 83,328	20.2 33.2	2.7 4.4	2.7 5.0	Decrease in lung and kidney weights	Pepelko et al. (1980b, 1981) Moorman et al. (1985)
Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/week 24 mo	0.84 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Increased rat and mouse lung weight at 7 mg/m^3 from 6 mo and at 2.5 mg/m^3 at 22 and 24 mo	Heinrich et al. (1995)

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Table 5-4. Effects of chronic exposures to diesel exhaust on organ weights and organ-to-body-weight ratios (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	Increased lung weight	Heinrich et al. (1995)
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Increase in lung weight was significant at 2 and 6 mg/m^3	Nikula et al. (1995)
Rat		0.8 2.5 6.98					Increased lung weight in rats and mice at 3.5 and 7.1 mg/m^3	Henderson et al. (1988)
Mouse		6.98 4.5						

[a]1 to 61 weeks of exposure.
[b]62 to 124 weeks of exposure.

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Table 5-5. Effects of diesel exhaust on pulmonary function of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MMD	7,280	11.5	1.5	0.8	No effect on pulmonary function	Lewis et al. (1989)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23-0.36 μm MMD	7,280	11.5	1.5	0.8	Decreased expiratory flow; no effect on vital or diffusing capacities	Lewis et al. (1989)
Rat, F344, M	20 h/day 5.5 days/week 87 weeks	1.5 0.19 μm MMD	14,355	7.0	0.5	—	Increased functional residual capacity, expiratory volume, and flow	Gross (1981)
Rat, Wistar, F	7-8 h/day 5 days/week 104 weeks	3.9 0.1 μm MMD	14,196-16,224	18.5	1.2	3.1	No effect on minute volume, compliance, or resistance	Heinrich et al. (1982)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	Decrease in vital capacity, residual volume, and diffusing capacity; increase in static deflation lung volume	Vinegar et al. (1980, 1981a,b)
Rat, F344, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.23–0.26 μm MMD	1,593 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Diffusing capacity, lung compliance reduced at 3.5 and 7.1 mg/m^3	Mauderly et al. (1988) McClellan et al. (1986)
Rat, F344, M, F; Hamster, Syrian, M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — —	— — —	— — —	Large number of pulmonary function changes consistent with obstructive and restrictive airway diseases at 6.6 mg/m^3 (no specific data provided)	Brightwell et al. (1986)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4.24 0.35 μm MMD	48,336	12.5	1.5	1.1	Significant increase in airway resistance	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24 0.35 μm MMD	56,392	12.5	1.5	1.1	Decrease in dynamic lung compliance; increase in airway resistance	Heinrich et al. (1986a)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[a] 12.0[b]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	Decrease in vital capacity, total lung capacity, and diffusing capacity after 2 years; no effect on expiratory flow	Pepelko et al. (1980b, 1981) Moorman et al. (1985)

[a]1 to 61 weeks exposure.
[b]62 to 124 weeks of exposure.

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Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 12-13 weeks	1.5 0.19 μm MDD	2,520-2,730	—	—	—	Inflammatory changes, increase in lung weight, increase in thickness of alveolar walls	Kaplan et al. (1982)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MDD	7,280	11.5	1.5	0.8	AM aggregation; no fibrosis, inflammation, or emphysema	Lewis et al. (1989)
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MDD	3,640	11.5	1.5	0.8	Multifocal histiocytosis, inflammatory changes, Type II cell proliferation, fibrosis	Bhatnagar et al. (1980) Pepelko (1982a)
Rat, Sprague-Dawley, M; Mouse, A/HEJ, M	8 h/day 7 days/week 39 weeks	6.0	13,104	—	—	—	Increase in lung protein content and collagen synthesis but a decrease in overall lung protein synthesis in both species; prolylhydroxylase activity increased in rats in utero	Bhatnagar et al. (1980) Pepelko (1982a)
Hamster, Chinese, M	8 h/day 5 days/week 26 weeks	6.0 12.0	6,240 12,480	— —	— —	— —	Inflammatory changes, AM accumulation, thickened alveolar lining, Type II cell hyperplasia,edema, increase in collagen	Pepelko (1982b)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 120 weeks	3.9 0.1 μm MDD	16,380-18,720	18.5	1.2	3.1	Inflammatory changes, 60% adenomatous cell proliferation	Heinrich et al. (1982)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8.3 0.71 μm MDD	21,663	50.0	4.0-6.0	—	Inflammatory changes, AM aggregation, alveolar cell hypertrophy, interstitial fibrosis, emphysema (diagnostic methodology not described)	Karagianes et al. (1981)
Rat, F344, F	8 h/day 7 days/week 104 weeks	4.9	28,538	7.0	1.8	13.1	Type II cell proliferation, inflammatory changes, bronchial hyperplasia, fibrosis	Iwai et al. (1986)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.23 μm MDD	1,592 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Alveolar and bronchiolar epithelial metaplasia in rats at 3.5 and 7.0 mg/m^3, fibrosis at 7.0 mg/m^3 in rats and mice, inflammatory changes	Mauderly et al. (1987a) Henderson et al. (1988)

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Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rats, SPF 344	7 h/day 5days/week 104 weeks	2 mg/m^3 coal dust (CD) 2 mg/m^3 DPM 1 mg/m^3 CD + 1 mg/m^3 DPM	—	—	—	—	• Assessed pharmacological responses of rat airway smooth muscle in vitro • Maximal contractile responses to acetylcholine of tissues from CD-, DPM-, and CD + DPM- exposed animals significantly increased; effects of CD and DPM were additive • Maximal relaxation response to isoproterenol increased significantly by CD + DPM exposure, but not by individual treatments • The results indicate that chronic exposure to CD, DPM, and CD + DPM produce differential modifications in the behavior of rat airway smooth muscle	Feden et al. (1985)
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/week 24 mo	0.8 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Bronchioalveolar hyperplasia, interstitial fibrosis in all groups. Severity and incidence increase with exposure concentration	Heinrich et al. (1995)
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	No increase in tumors. Noncancer effects not discussed	
Mouse		4.5					No increase in tumors Noncancer effects not discussed	
Rat, M, F, F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11[a] 0.41[a] 1.08[a] 2.31[a] 3.72[b]	1,373 5,117 13,478 28,829 46,336	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Inflammatory changes Type II cell hyperplasia and lung tumors seen at >0.4 mg/m^3; shortening and loss of cilia in trachea and bronchi	Research Committee for HERP Studies (1988)

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Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, NMRI, F	19 h/day 5 days/week 120 weeks	4.24	48,336	12.5	1.5	1.1	Inflammatory changes, bronchioloalveolar hyperplasia, alveolar lipoproteinosis, fibrosis	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24	56,392	12.5	1.5	1.1	Thickened alveolar septa; AM aggregation; inflammatory changes; hyperplasia; lung tumors	Heinrich et al. (1986a)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 104 weeks	0.25 0.75 1.5 6.0	2,860 8,580 17,160 68,640	— — — —	— — — —	— — — —	Minimal response at 0.25 and ultrastructural changes at 0.75 mg/m^3; thickened alveolar membranes; cell proliferation; fibrosis at 6.0 mg/m^3; increase in PMN at 0.75 mg/m^3 and 1.5 mg/m^3	Barnhart et al. (1981, 1982) Vostal et al. (1981) Wallace et al. (1987)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[c] 12.0[d]	41,664 83,328	20.2 33.2	2.7 4.4	2.1 5.0	Inflammatory changes, AM aggregation, bronchiolar epithelial metaplasia, Type II cell hyperplasia, peribronchiolar fibrosis	Plopper et al. (1983) Hyde et al. (1985)
Rat, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	AM hyperplasia, epithelial hyperplasia, inflammation, septal fibrosis, bronchoalveolar metaplasia	Nikula et al. (1995)
Mouse, CD-1, M,F	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 μm MDD	1,274 12,740 25,844	3 17 30	0.1 0.3 0.7	— — —	Exposure-related increase in lung soot, pigment-laden macrophages, lung lesions. Bronchiolization in alveolar ducts at 7.1 mg/m^3	Mauderly et al. (1996)

[a]Light-duty engine.
[b]Heavy-duty engine.
[c]1 to 61 weeks exposure.
[d]62 to 124 weeks of exposure.

AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals

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Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
			Alveolar macrophage status					
Guinea Pig, Hartley	20 h/day 5.5 days/week 8 weeks	0.25 1.5 0.19 μm MDD	220 1,320	2.9 7.5	— —	— —	No significant changes in absolute numbers of AMs	Chen et. al. (1980)
Rat, F344, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 μm MDD	7,280	11.5	1.5	0.81	Little effect on viability, cell number, oxygen consumption, membrane integrity, lyzomal enzyme activity, or protein content of AMs; decreased cell volume and ruffling of cell membrane and depressed luminescence of AM	Castranova et al. (1985)
Rat, F344, M	20 h/day 5.5 days/week 26, 48, or 52 weeks	0.25[a] 0.75[a] 1.5[b] 0.19 μm MDD	715-8,580	2.9 4.8 7.5	— — —	— — —	AM cell counts proportional to concentration of DPM at 0.75 and 1.5 mg/m^3; AM increased in lungs in response to rate of DPM mass entering lung rather than total DPM burden in lung; increased PMNs were proportional to inhaled concentrations and/or duration of exposure; PMNs affiliated with clusters of aggregated AM rather than DPM	Strom (1984) Vostal et al. (1982)
Rat F344/Crl, M, F Mouse, CD, M, F	7 h/day 5 days/week 104 weeks (rat), 78 weeks (mouse)	0.35 3.5 7.0 0.25 μm MDD	1,274[c] 12,740[c] 25,480[c]	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Significant increases of AM in rats and mice exposed to 7.0 mg/m^3 DPM for 24 and 18 mo, respectively, but not at concentrations of 3.5 or 0.35 mg/m^3 DPM for the same exposure durations; PMNs increased in a dose-dependent fashion in both rats and mice exposed to 3.5 or 7.0 mg/m^3 DPM and were greater in mice than in rats	Henderson et al. (1988)
Rat, Wistar, F	18 h/day 5 days/week 24 mo	0.8 2.5 7.1	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.1 3.4	— — —	Changes in differential cell counts in lung lavage	Heinrich et al. (1995)
Rat, F344/Crl, M	7 h/day 5 days/week 24 mo	3.49	12,704	9.8	1.2	—	Significantly reduced AM in lavage at 24 mo	Mauderly et al. (1990a)

Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
				Clearance				
Rat, M, F	7 h/day 5 days/week 12 weeks	0.2 1.0 4.5 0.25μm MDD	84 420 1,890	— — —	— — —	— — —	Evidence of apparent speeding of tracheal clearance at the 4.5 mg/m^3 level after 1 week of ^{99m}Tc macroaggregated-albumin and reduced clearance of tracer aerosol in each of the three exposure levels at 12 weeks; indication of a lower percentage of ciliated cells at the 1.0 and 4.5 mg/m^3 levels	Wolff and Gray (1980)
Rat, Wistar, F	18 h/day 5 days/week 24 mo	0.8 2.5 7.1	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Significant increase in clearance half-time of inhaled labeled aerosols in all groups at 3-18 mo	Heinrich et al. (1995)
Rat, F344, M, developing 0-6 mo adult 6-12 mo	7 h/day 5 days/week 6 mo	3.55	3,321	7.9	9.5		Clearance of 2 μm, aluminosilicate particles. Half-time significantly increased in adult, not different in developing rats	Mauderly et al. (1987b)
Rat, F344, M, F	7 h/day 5 days/week 18 weeks	0.15 0.94 4.1 <0.5 μm MDD	94.5 592 2,583	— — —	— — —	— — —	Lung burdens of DPM were concentration-related; clearance half-time of DPM almost double in 4.1 mg/m^3 group compared to 0.15 mg/m^3 group	Griffis et al. (1983)
Rat, F344, M	7 h/day 5 days/week 26-104 weeks	2.0 0.23-0.36 μm MDD	1,820-7,280	11.5	1.5	0.8	No difference in clearance of $^{59}Fe_3O_4$ particles 1 day after tracer aerosol administration; 120 days after exposure tracer aerosol clearance was enhanced; lung burden of DPM increased significantly between 12 and 24 mo of exposure	Lewis et al. (1989)
Rat, Sprague-Dawley, M	4-6 h/day 7 days/week 0.1 to 14.3 weeks	0.9 8.0 17.0	2.5-10,210	— — —	5.0 2.7 8.0	0.2 0.6 1.0	Impairment of tracheal mucociliary clearance in a concentration-response manner	Battigelli et al. (1966)

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Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.25 μm MDD	1,593 15,925 31,850	2.9 16.5 29.7	0.1 0.3 0.7	— — —	No changes in tracheal mucociliary clearance after 6, 12, 18, 24, or 30 mo of exposure; increases in lung clearance half-times as early as 6 mo at 7.0 mg/m^3 level and 18 mo at 3.5 mg/m^3 level; no changes seen at 0.35 mg/m^3 level; after 24 mo of diesel exposure, long-term clearance half-times were increased in the 3.5 and 7.0 mg/m^3 groups	Wolff et al. (1987)
Rat, F344/Crl, M	7 h/day 5 days/week 24 mo	3.49	12,704	9.8	1.2	—	Doubling of long-term clearance half-time for clearance of 1.0 μm aluminosilicate particles. Less effect on clearance in animals with experimentally induced emphysema	Mauderly et al. (1990a)
			Microbial-induced mortality					
Mice CD-1, F	7 h/day 5 days/week 4, 12, or 26 weeks	2.0 0.23–0.36 μm MDD	280-1,820	11.5	1.5	0.8	Mortality similar at each exposure duration when challenged with Ao/PR/8/34 influenza virus; in mice exposed for 3 and 6 mo, but not 1 mo, there were increases in the percentages of mice having lung consolidation, higher virus growth, depressed interferon levels, and a fourfold reduction in hemagglutinin antibody levels	Hahon et al. (1985)
Mice, CR/CD-1, F	8 h/day 7 days/week 2 h up to 46 weeks	5.3 to 7.9	11-20,350	19 to 22	1.8 to 3.6	0.9 to 2.8	Enhanced susceptibility to lethal effects of *S. pyogenes* infections at all exposure durations (2 and 6 h; 8, 15, 16, 307, and 321 days); inconclusive results with *S. typhimurium* because of high mortality rates in controls; no enhanced mortality when challenged with A/PR8-3 influenza virus	Campbell et al. (1980, 1981)

[a]Chronic exposure lasted 52 weeks.
[b]Chronic exposure lasted 48 weeks.
[c]Calculated for 104-week exposure.
DPM = Diesel particulate matter.
AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

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Table 5-8. Effects of inhalation of diesel exhaust on the immune system of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 4 or 8 weeks	1.5 0.19 μm MDD	660 or 7,280	7.5	—	—	No alterations in numbers of B, T, and null lymphocytes or cell viability among lymphocytes isolated from tracheobronchial lymph nodes, spleen, or blood	Dziedzic (1981)
Rat, F344, M	7 h/day 5 days/week 52 or 104 weeks	2.0 0.23–0.36 μm MDD	3,640 or 7,280	11.5	1.5	0.8	Neither humoral immunity (assessed by enumerating antibody-producing cells) nor cellular immunity (assessed by the lymphocyte blast transformation assay) were markedly affected	Mentnech et al. (1984)
Rat, F344; Mouse, CD-1	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 μm MDD	1,274 12,740 25,480	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Total number of anti-sheep red blood cell IgM AFC in the lung-associated lymph nodes was elevated in rats exposed to 3.5 or 7.0 mg/m^3 DPM (no such effects in mice); total number of AFC per 10^6 lymphoid cells in lung-associated lymph nodes and level of specific IgM, IgG, or IgA in rat sera were not altered	Bice et al. (1985)
Mouse, BALB/C, M	12 h/day, 7 days/week, 3 weeks Mice administered OA intranasally before, immediately after, and 3 weeks after exposure	3.0 6.0 0.4 μm	756 1,512	— —	2.8 4.1	1.7 2.7	Spleen weights in mice exposed to diesel exhaust (6 mg/m^3) increased significantly. Serum anti-OA IgE antibody titers in mice exposed to 6 mg/m^3 significantly higher than control. Antigen-stimulated IL-4 and IL-10 production increased while IFN-g production decreased significantly in spleen cells from diesel exhaust-exposed (6 mg/m^3) mice stimulated with OA in vitro. Diesel exhaust inhalation may affect antigen-specific IgE antibody production through alteration of the cytokine network.	Fujimaki et al. (1997)
Mouse, C3H/Hen, M	12 h /day, for 12 weeks. Before exposure mice injected IP with OA. After 3 weeks and every 3 weeks thereafter, mice challenged with OA aerosol.	1.0 3.0	1,008 3,024	—	1.42 4.02	0.87 1.83	Diesel exhaust + antigen challenge induced airway hyperresponsiveness and inflammation with increased eosinophils, mast cells, and goblet cells. Diesel exhaust alone induced airway hyperresponsiveness, but not eosinophilic infiltration or increased goblet cells. Diesel exhaust inhalation enhanced airway hyperresponsiveness and airway inflammation caused by OA sensitization.	Miyabara et al. (1998a)

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Table 5-8. Effects of inhalation of diesel exhaust on the immune system of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, C3H/HeN, M	12 h/day, for 5 weeks. After 7 days mice injected IP with OA. At end of exposure mice challenged with OA aerosol for 15 minutes.	3.0	1,260	—	4.08	1.26	Diesel exhaust alone increased neutrophils and macrophages in BAL fluid; after diesel exhaust + OA challenge eosinophils increased. OA alone increased eosinophils but the increase was enhanced by diesel exhaust. Diesel exhaust + OA, but not diesel exhaust alone, increased goblet cells, respiratory resistance, production of OA-specific IgE and Ig1 in the serum, and overexpression of IL-5 in lung tissue.	Miyabara et al. (1998b)
Mouse, ICR (murine model of allergic asthma)	12 h/day, 7days/week, 40 weeks. After 16 weeks sensitized to OA and challenged with OA aerosol for 6 min, at 3-week intervals during the last 24 weeks of exposure.	0.3 1.0 3.0	1,008 3,360 10,080	—	—	—	Diesel exhaust exposure enhanced allergen-related recruitment to the submucosal layers of the airways and the bronchoalveolar space, and increased GM-CSF and IL-5 in the lung in a dose-dependent manner. Increases in eosinophil recruitment and local cytosine expression accompanied by goblet cell proliferation in the bronchial epithelium and airway hyperresponsiveness to inhaled acetylcholine. Mice exposed to clean air or DE without allergen provocation showed no eosinophil recruitment to the submucosal layers of the airways nor to the bronchoalveolar space, and few goblet cells in the bronchial epithelium. Daily inhalation of DE may enhance allergen-related respiratory diseases such as allergic asthma, and effect may be mediated by the enhanced local expression of IL-5 and GM-CSF.	Takano et al. (1998a)

DPM = Diesel particulate matter.
AFC = Antibody-forming cells.

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Table 5-9. Effects of diesel particulate matter on the immune response of laboratory animals

Model	Treatment	Effects	Reference
Mouse, BDFI, F		Intranasally delivered doses of DPM as low as 1 mg exerted an adjuvant activity for IgE antibody production.	Takafuji et al. (1987)
Mouse, ICR, w/w^{-}, M	Intratracheal instillation of DPM, once/week for 16 weeks	Infiltration of inflammatory cells, proliferation of goblet cells, increased mucus secretion, respiratory resistance, and airway constriction. Increased eosinophils in the submucosa of the proximal bronchi and medium bronchioles. Eosinophil infiltration suppressed by pretreatment with PEG-SOD. Bound sialic acid, an index of mucus secretion, in bronchial alveolar lavage fluids increased, but was suppressed by PEG-SOD. Increased respiratory resistance suppressed by PEG-SOD. Oxygen radicals produced by instilled DPM may cause features characteristic of bronchial asthma in mice.	Sagai et al. (1996)
Mouse, A/J, M	Mice immunized intranasally with Der f II + pyrene, or Der f II + DPM 7 times at 2-week intervals	IgE antibody responses to Der f II enhanced in mice immunized with Der f II+ pyrene or Der f II + DPM compared with Der f II alone. Response was dose related. DPM and pyrene contained in DPM have adjuvant activity on IgE and IgG1 antibody production in mice immunized with house dust mite allergen.	Suzuki et al. (1996)
Mouse, BDF_1, M	Mice were administered 25 mg of each of 5 fine particles (Kanto loam dust, fly ash, CB, DPM, and aluminum hydroxide [alum]) intranasally and exposed to aerosolized Japanese cedar pollen allergens (JCPA) for intervals up to 18 wk	Measurements were made of JCPA-specific IgE and IgG antibody titers, the protein-adsorbing capacity of each type of particle, and nasal rubbing movements (a parameter of allergic rhinitis in mice). The increases in anti-JPCA IgE and IgG antibody titers were significantly greater in mice treated with particles and aerosolized JCPA than in mice treated with aerosolized JCPA alone. In a subsequent experiment, the mice received the particles as before, but about 160,000 grains of Japanese cedar pollen (JCP) were dropped onto the tip of the nose of each mouse twice a week for 16 wk. After 18 wk there were no significant differences in the anti-JCPA IgE and IgG production, nasal rubbing, or histopathological changes. The workers concluded that the nature of the particle, the ability of the particle to absorb antigens, and/or particle size is not related to the enhancement of IgE antibody production or symptoms of allergic rhinitis. However, IgE antibody production did appear to occur earlier in mice treated with particles than in mice immunized with allergens alone.	Maejima et al. (1997)
Mouse, BALB/C, nu/nu, F	Inoculated OA with DPM or CB into hind footpad measured response using popliteal lymph node assay	Increased response (increased weight, cell numbers, cell proliferation) and longer response observed with DPM and OA, compared to DPM or OA alone. Response was specific and not an unspecific inflammatory response. CB was slightly less potent than DPM. Nonextractable carbon core contributes substantially to adjuvant activity of DPM.	Løvik et al. (1997)
Mouse, BALB/cA, F	Intranasal administration of DPM. Mice immunized with OA or OA combined with DPM or CB	Increased response to antigen in animals receiving DPM or CB. Increased number of responding animals and increased serum anti OA IgE antibody. Both DPM and CB have adjuvant activity for IgE production. DPM response more pronounced than CB, indicating both organic matter adsorbed to DPM and the nonextractable carbon core responsible for adjuvant activity.	Nilsen et al. (1997)
Mouse, ICR, M	Intratracheal instillation of OA, DPM, or OVA and DPM combined, once/week for 6 wk	Respiratory resistance (Rrs) measured 24 h after the final instillation. Rrs after acetylcholine challenge was significantly greater in the mice treated with OVA and DPM than other treatments. DPM can enhance airway responsiveness associated with allergen exposure.	Takano et al. (1998b)

OA - Ovalbumin.
DPM - Diesel particulate matter.
CB - Carbon black.

PEG-SOD - Polyethyleneglycol-conjugated superoxide dismutase.
IL-4 - Interleukin-4.
IL-5 - Interleukin-5.
IL-10 - Interleukin-10.
IFN - Interferon-g.
GM-CSF -Granulocyte-colony stimulating factor.
IP - Intraperitoneally.

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Table 5-10. Effects of exposure to diesel exhaust on the liver of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 52 weeks	2.0 0.23–0.36 μm MDD	3,640	12.7	1.6	0.83	No changes in absolute liver weight or liver/body weight ratio	Green et al. (1983)
Hamster, Syrian	7-8 h/day 5 days/week 22 weeks	4.0 8.0 11.0	3,080-9,680	12.0 19.0 25.0	0.5 1.0 1.5	3.0 6.0 7.0	Enlarged sinusoids, with activated Kupffer's cells and slight changes of nuclei; fatty deposits; mitochondria, loss of cristae and pleomorphic character; gap junctions between hepatocytes had wide range in structural diversity	Meiss et al. (1981)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[a] 12.0[b]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	No change in the absolute liver weight	Plopper et al. (1983)

[a]1 to 61 weeks of exposure.
[b]62 to 124 weeks of exposure.

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Table 5-11. Effects of exposure to diesel exhaust on the hematological and cardiovascular systems of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2 0.23–0.36 μm MDD	7,280	11.5	1.5	0.8	Increased MCV	Lewis et al. (1989)
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2 0.23–0.36 μm MDD	7,280	11.5	1.5	0.8	Increase in banded neutrophils; no effect on heart or pulmonary arteries	Lewis et al. (1989) Vallyathan et al. (1986)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3[a] 6.8[b]	7,056 7,616	17.4 16.7	2.3 2.9	2.1 1.9	No effect on heart mass or ECG; small decrease in heart rate (IE only)	Wiester et al. (1980)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 75 weeks	3.9 0.1 μm MDD	10,238-11,700	18.5	1.2	3.1	At 29 weeks, lower erythrocyte count; increased MCV; reduced leukocyte count	Heinrich et al. (1982)
Rat, F344; Guinea Pig, Hartley	20 h/day 5.5 days/week 78 weeks	0.25 0.75 1.5 0.19 μm MDD	2,145 6,435 12,870	3.0 4.8 6.9	0.11 0.27 0.49	— — —	No changes in heart mass or hematology at any exhaust level or duration of exposure in either species	Penney et al. (1981)
Rat, Wistar, M	6 h/day 5 days/week 78 weeks	8.3 0.71 μm MDD	19,422	50.0	4-6	—	3% increase in COHb	Karagianes et al. (1981)
Rat, F3444/Jcl, M, F	16 h/day 6 days/week 130 weeks	0.11[c] 0.41[c] 1.08[c] 2.31[c] 3.72[d] 0.1 μm MDD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	At higher concentrations, RBC, Hb, Hct slightly elevated; MCV and mean corpuscular hemoglobin and concentration were lowered	Research Committee for HERP Studies (1988)
Rat, F344	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Increases in RBC, Hb, Hct, and WBC, primarily banded neutrophils; suggestion of an increase in prothrombin time; increased heart/body weight and right ventricular/heart ratios and decreased left ventricular contractility in 6.6 mg/m^3 group	Brightwell et al. (1986)
Cat, Inbred, M	8 h/day 7 days/week 124 weeks	6.0[e] 12.0[f]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	Increases in banded neutrophils; significant at 12 mo, but not 24 mo	Pepelko and Peirano (1983)

[a]Nonirradiated diesel exhaust.
[b]Irradiated diesel exhaust.
[c]Light-duty engine.
[d]Heavy-duty engine.
[e]1 to 61 weeks of exposure.
[f]62 to 124 weeks of exposure.

Key: MCV = Mean corpuscular volume.

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Table 5-12. Effects of chronic exposures to diesel exhaust on serum chemistry of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23 0.36 μm MDD	7,280	11.5	1.5	0.8	Decreased phosphate, LDH, SGOT, and SGPT; increased sodium in females but not males	Lewis et al. (1989)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 75 weeks	3.9 0.1 μm MDD	10,238-11,700	18.5	1.2	3.1	After 29 weeks, increases in SGOT, LDH, alkaline phosphatase, gamma-glutamyl transferase, and BUN	Heinrich et al. (1982)
Rat, F344/JcL, M, F	16 h/day 6 days/week 130 weeks	0.11[a] 0.41[a] 1.08[a] 2.31[a] 3.72[b] 0.19–0.28 μm MDD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 3.96 7.10 3.00	0.38 1.06 2.42 4.70 4.57	Lower cholinesterase activity in males in both the light-and heavy-duty series and elevated gamma globulin and electrolyte levels in males and females in both series	Research Committee for HERP Studies (1988)
Rat, F344; Hamster, Syrian	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Rats, 6.6 mg/m^3, reduction in blood glucose, blood proteins, triglycerides, and cholesterol; increase in BUN, alkaline phosphate alamine, and aspartate aminotransferases (SGPT and SGOT); hamsters, 6.6 mg/m^3, decrease in potassium, LDH, aspartate amino-transferase; increase in albumin and gamma-glutamyl transferase	Brightwell et al. (1986)
Cat inbred, M	8 h/day 7 days/week 124 weeks	6.0[c] 12.0[d]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	BUN unaltered; SGOT and SGPT unaffected; LHD increase after 1 year of exposure	Pepelko and Peirano (1983)

[a]Light-duty engine.
[b]Heavy-duty engine.
[c]1 to 61 weeks of exposure.
[d]62 to 124 weeks of exposure.

Key: LDH = Lactate dehydrogenase.
SGOT = Serum glutamic-oxaloacetic transaminase.
BUN = Blood urea nitrogen.
SGPT = Serum glutamic-pyruvic transaminase.

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Table 5-13. Effects of chronic exposures to diesel exhaust on microsomal enzymes of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × t ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M	—	—	—	—	—	—	Intratracheal administration of DPM extract required doses greater than 6 mg/m^3 before the lung AHH was barely doubled; liver AHH activity was unchanged	Chen (1986)
Mouse, CD-1, F	7 h/day 5 days/week 4 weeks	2.0 0.2–0.36 μm mdd	280	11.5	1.5	0.8	Mice inoculated intranasally with influenza virus had smaller increases in ethylmorphine demethylase activity on days 2 to 4 postvirus infection and abolition of day 4 postinfection increase in NADPH-dependent cytochrome c reductase	Rabovsky et al. (1986)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 1-7 weeks	6.3	882-6,174	17.4	2.3	2.1	AHH induction occurred in lung, liver, and prostate gland but not in testes; maximum significant activities occurred at different times; liver has greatest overall activity, percent increase highest in prostate; expoxide hydrase activity was unaffected	Lee et al. (1980)
Rat, F344, M	20 h/day 5.5 days/week 4, 13, 26, or 39 weeks	0.75 1.5 0.19 μm mdd	330-6,435	4.8 7.5	— —	— —	Inhalation exposure had no significant effect on liver AHH activity; lung AHH activity was slightly reduced after 6-mo exposure to 1.5 mg/m^3 DPM; an ip dose of dp extract, estimated to be equivalent to inhalation exposure, had no effect on AHH activity in liver and lungs; cyt. P-50 was unchanged in lungs and liver following inhalation or ip administration	Chen and Vostal (1981)
	20 h/day 5.5 days/week 4, 13, 26, or 39 weeks	0.75 1.5 0.19 μm mdd	330-6,435	4.8 7.5	— —	— —		
Rat, F344, F	7 h/day 5 days/week 12, 26, or 104 weeks	2.0 0.23-0.36 μm mdd	840-7,280	11.5	1.5	0.8	No effect on B[*a*]p hydrolase or 7-exthoxycoumarin deethylase activities in the liver	Rabovsky et al. (1984)
Rat, F344, M	20 h/day 5.5 days/week 8-53 weeks	0.25 1.5 0.19 μm mdd	220-8,745	2.9 7.5	— —	— —	After 8 weeks, no induction of cyt. P-450, cyt. P-448, or NADPH-dependent cyt. c reductase; after 1 year of exposure, liver microsomal oxidation of B[*a*]p was not increased; 1 year of exposure to either 0.25 or 1.5 mg/m^3 DPM impaired lung microsomal metabolism of B[*a*]p	Navarro et al. (1981)
Mouse, A/J, M	8 days/week 7 days/week 26 or 35 weeks	6.0	17.4	17.4	2.3	2.1	No differences in lung and liver AHH activities and liver P-448, P-450 levels	Pepelko and Peirano (1983)

AHH = aryl hydrocarbon hydroxylase.
B[*a*]p = benzo[*a*]pyrene.

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Table 5-14. Effects of chronic exposures to diesel exhaust on behavior and neurophysiology

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, Sprague-Dawley, M	8 h/day 7 days/week 1-4 weeks	6	336-1,344	19	2.5	1.8	Somatosensory and visual evoked potentials revealed longer pulse latencies in pups exposed neonatally	Laurie and Boyes (1980, 1981)
Rat, Sprague Dawley, F	20 h/day 7 days week 6 weeks	6	5,040	19	2.5	1.8	Reduction in adult SLA and in neonatal pivoting	Laurie et al. (1978)
Rat, Sprague-Dawley, F	8 or 20 h/day 7 days/week 3, 4, 6, or 16 weeks	6	1,008-13,440	19	2.5	1.8	Reduction in SLA in adults; neonatal exposures for 20 or 8 h/day caused reductions in SLA. Neonatal exposures for 20 h/day for 17 days resulted in a slower rate of a bar-pressing task to obtain food	Laurie et al. (1980)

SLA = Spontaneous locomotor activity.

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Table 5-15. Effects of chronic exposures to diesel exhaust on reproduction and development in laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, [C57BL]/ 6XC3H]F_1, M	5 days	50, 100, or 200 mg/kg in corn oil; i.p. injection	—	—	—	—	Dose-related increase in sperm abnormalities; decrease in sperm number at highest dose; testicular weights unaffected	Quinto and De Marinis (1984)
Rat, Sprague-Dawley, F	8 h/day 7 days/week 1.7 weeks	6	571	20	2.7	2.1	No signs of maternal toxicity or decreased fertility; no skeletal or visceral teratogenic effects in 20-day-old fetuses	Werchowski et al. (1980a) Pepelko and Peirano (1983)
Rabbit, New Zealand Albino, F	8 h/day 7 days/week 1.9 weeks	6	638	20	2.7	2.1	No adverse effects on maternal weight gain or fertility; no skeletal or visceral teratogenic effects in the fetuses	Werchowski et al. (1980a) Pepelko and Peirano (1983)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2	7,280	11.5	1.5	0.8	No effects on sperm motility, velocity, density, morphology, or incidence of abnormalities	Lewis et al. (1989)
Mouse, A/Strong, M	8 h/day 7 days/week 31 or 38 weeks	6	10,416-12,768	20	2.7	2.1	No effect on sperm morphology; high rate of spontaneous sperm abnormalities may have masked small effects	Pereira et al. (1981)
Mouse, CD-1, M, F	8 h/day 7 days/week 6 to 28 weeks	12	4,032-18,816	33	4.4	5.0	Overall fertility and survival rates were unaffected in the three-generation reproductive study; only consistent change noted, an increase in lung weights, was diagnosed as anthracosis	Pepelko and Peirano (1983)

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Table 5-16. Composition of exposure atmospheres in studies comparing unfiltered and filtered diesel exhaust[a]

Species/sex	Exposure[b] period		Particles (mg/m^3)	C × t ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, Wistar, F; Hamster, Syrian	7 h/day 5 days/week 104 weeks	Uf F C	3.9 — —	14,196	18.5 18.0 —	1.2 1.0 —	3.1 2.8 —	No effect on pulmonary function or heart rate in rats; increases in pulmonary adenomatous proliferations in hamsters, UF significantly higher than F or C	Heinrich et al. (1982)
Rat, F344, F	8 h/day 7 days/week 104 weeks	Uf F[c] C	4.9 — —	28,538	7.0 — —	1.8 — —	13.1 — —	Body weight decrease after 6 mo in UF, 18 mo in f; lung/body rate weight rate higher in both groups at 24 mo; at 2 years, fibrosis and epithelial hyperplasia in lungs of uf; nominal lung and spleen histologic changes	Iwai et al. (1986)
Rat, F344, M, F; Hamster, Syrian, M, F	16 h/day 5 days/week 104 weeks	Uf Uf Uf F[d] C	0.7 2.2 6.6 — —	5,824 18,304 54,912	— — 32.0 32.0 1.0	— — — — —	— — — — —	Uf: elevated red and white cell counts, hematocrit and hemoglobin; increased heart/body weight and right ventricular/heart weight ratios; lower left ventricular contractility; changes in blood chemistry; obstructive and restrictive lung disease; F: no effects	Brightwell et al. (1986)
Rat, Wistar, F; Hamster, Syrian, F; Mouse NMRI, F	19 h/day 5 days/week 120 to 140 weeks	Uf F[d] C	4.24 — —	48,336 56,392	12.5 11.1 0.16	1.5 1.2 —	3.1 1.02 —	Uf: decreased body wt in rats and mice but not hamsters; increased mortality, mice only; decreased lung compliance and increased airway resistance, rats and hamsters; species differences in lung lavage enzymes and cell counts and lung histopathology and collagen content, most pronounced in rats; F: no effect on glucose-6-phosphate dehydrogenase, total protein, and lung collagen	Heinrich et al. (1986a)
Mouse, NMRI, F, C57BL/6N, F	18 h/day 5 days/week 23 mo (NMRI) 24 mo (C57BL/6N)	Uf F C	4.5 0.01 0.01	40,365	14.2 14.2 0.2	2.3 2.9 0.01	2.8 2.4 0.1	Uf: increased lung wet weight starting at 3 mo F: no noncancer effects reported	Heinrich et al. (1995)

[a]Man values.
[b]UF= unfiltered whole exhaust, F = filtered exhaust, C = control.
[c]Reported to have the same component concentrations as the unfiltered, except particles were present in undetectable amounts.
[d]Concentrations reported for high concentration level only.

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