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Health Assessment Document for Diesel Emissions

Workshop Review Draft

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Sections: 1 Thru 10

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U.S. Environmental Protection Agency
Office of Reasearch and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

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1. SUMMARY

1.1. INTRODUCTION

Diesel engines are used to power ships, heavy machinery, locomotives, and heavy duty trucks, as well as a small number of light-duty passenger cars and trucks. Cuddihy et al. (1984) estimated that about 2 percent of the light-duty vehicles in the United States were equipped with diesel engines.

Since passage of the Clean Air Act of 1963 and the Motor Vehicle Air Pollution Control Act of 1965, efforts have been made by the federal government to limit pollutant emissions from mobile sources to levels that will be protective of human health and the environment. Regulation of emissions from gasoline- and diesel-powered vehicles falls under the authority of EPA's Office of Air and Radiation, Office of Mobile Sources. EPA particulate emission standards for light-duty diesel (LDD) vehicles and trucks were promulgated in 1980 and became effective in 1982 (U.S. EPA, 1980). In 1987, the particulate emission standard for LDD vehicles became 0.20 g/mi, and the standard for LDD trucks became 0.26 g/mi. For heavy-duty diesel (HDD) vehicles, a particulate emission standard of 0.6 gram/brake horsepower-hour (g/bhp-h) took effect in 1988. In 1991, the standard becomes 0.10 g/bhp-h for urban buses and 0.26 g/bhp-h for all other HDD vehicles. Effective in 1994, the emission standard for all HDD vehicles will be 0.10 g/bhp-h (Carey, 1987). A summary of gaseous and particulate emission standards for new LDD and HDD vehicles is given in Appendix A.

The continued use of diesel vehicles has raised concerns regarding the potential health and environmental effects associated with diesel exhaust. Polycyclic aromatic hydrocarbons (PAH) are adsorbed onto the diesel exhaust particles, and several of these, such as benzo[a]pyrene and 1-nitropyrene, are known or potential human carcinogens. As early as 1955, it was reported that repeated skin applications of organic solvent extracts of exhaust particles resulted in an increased incidence of skin tumors in mice. In 1979, such extracts were shown to be mutagenic in the Ames *Salmonella* assay, a useful bioassay for

indicating possible carcinogenicity. Since then, the genotoxicity of exhaust extracts has been demonstrated in a number of *in vitro* and *in vivo* assays. Much of the reported mutagenicity of diesel exhaust extracts has been attributed to nitrated PAHs, and particularly to nitropyrenes, dinitropyrenes, and nitrohydroxypyrenes.

Suggestive evidence for a carcinogenic effect of inhaled diesel exhaust in animals was first reported in 1981, in studies demonstrating an increased incidence of pulmonary adenomas in mice treated with the tumor promotor urethane and subsequently exposed to diesel exhaust. Recent animal studies have since demonstrated a clear association between chronic inhalation of diesel exhaust and increased incidences of lung tumors.

Evidence for the potential carcinogenicity of diesel exhaust in humans is limited; however, a few recent studies have indicated a small but significant increased risk of lung cancer in occupationally exposed workers.

In addition to the potential carcinogenicity of diesel exhaust, there has also been some concern that diesel particulate matter may contribute to other health problems, especially those associated with the respiratory tract. Respirable particles such as those in diesel exhaust have been implicated as etiological factors in various types of chronic lung disease. They may also increase the lung's susceptibility to bacterial and viral infections, aggravate preexisting diseases such as bronchitis or emphysema, or aggravate specific respiratory conditions such as bronchial asthma (U.S. EPA, 1986). There is also some evidence for adverse behavioral and neurological effects. Other components of diesel exhaust, such as sulfur dioxide, nitrogen dioxide, formaldehyde, acrolein, and sulfuric acid may contribute to some of these potential health effects.

Several EPA analyses have been conducted to assess the economic, environmental, and health impacts of the current and proposed changes in the diesel emissions standards, especially as they pertain to particulate matter (U.S. EPA, 1983; Carey, 1987; Carey and Somers, 1988). These analyses indicate that emissions of diesel particulate matter in the United States in 1986 totaled 274,000 metric tons, or about 3.9 percent of the total suspended particulate emissions from all sources (Carey, 1987). As a result of new and proposed mobile source emission standards, diesel particulate emissions were projected to drop to 125,000 to 154,000 metric tons/year in 1995. The annual mean exposure of the U.S.

population to diesel particulate matter in 1986 was estimated to be 2.6 $\mu g/m^3$ (Carey, 1987). The exposure level for 1995 was projected to drop to 1.2 to 1.6 $\mu g/m^3$ as a result of the new and proposed emission standards.

EPA has evaluated the carcinogenic risks associated with the continued use of diesel-powered motor vehicles. The calculations were based on the exposure estimates given above and also on estimates of the carcinogenicity of diesel exhaust. In 1983, EPA calculated the carcinogenic potency of diesel particulate matter by the comparative potency method (Albert et al., 1983; see also U.S. EPA, 1983). The upper confidence limit on unit risk was found to be 0.2 to 1.0×10^{-4} per $\mu g/m^3$ of particulate matter for a lifetime exposure. Using these data, EPA estimated that the annual lung cancer incidence in the U.S. population in 1986 due to diesel exhaust particulate matter ranged from 178 to 860 (Carey, 1987). It was also estimated that the incidence rate in 1995 would fall between 92 and 443. The latter figure took into account the more stringent emissions standards and also allowed for a range of small to large increases in the use of diesel engines in light-duty vehicles.

Since EPA's initial evaluation of the carcinogenic risks associated with diesel exhaust, additional animal carcinogenicity data have become available. The purpose of this report is to reevaluate the carcinogenic potency of diesel particulate matter in light of the new data from these animal studies, as well as to reexamine the evidence available from human epidemiological studies. Additional issues that are addressed include the interrelationship between carcinogenic effects and rates of deposition and clearance of the exhaust particles from the lungs, and the significance of potentially carcinogenic organic compounds adsorbed to the exhaust particles and their subsequent desorption and bioavailability. Also included is an overview of the potential noncarcinogenic health effects associated with exposure to diesel exhaust.

1.2. COMPOSITION OF DIESEL EXHAUST

The purpose of this chapter is to provide a basic understanding of how the Diesel engine contributes to the atmospheric cheical inventory attributed to mobile sources. Its five elements will provide information which will serve to put those chapters that follow in the proper context.

1.2.1. INTRODUCTION

1.2.1.1. Diesel Engine — What It Is and Why It Is Used.

1. Design Aspects — Two and four stroke cycle; naturally aspirated and turbocharged; fuel and lubricant systems
2. Typical Uses — Light-duty vehicles; line—haul trucks; buses; off—road equipment; locomotives

1.2.1.2. Fundamentals of Diesel Combustion — Heterogeneous with Excess Air.

1. Geometric Variables — Compression ratio; direct and indirect injection; injection timing
2. Operating Variables — Load; speed; lubricant; fuel

1.2.2. OVERVIEW OF POLLUTANTS AND POLLUTANT FORMATION

1.2.2.1. Gas Phase Emissions

1. Oxides of Nitrogen Formation
2. Hydrocarbon and Carbon Monoxide Formation

1.2.2.2. Carbon Formation and Emission

1. In Flames
2. Physical Characteristics of Emitted Particles

1.2.2.3. Gas—to—Particle Conversions

1. Organics———> Condensation — Absorption
2. Acids———> Oxidation — Condensation

1.2.2.4. Mutagens

1. Role of Nitrogen and its Oxides
2. Identification of Emitted Mutagens

1.2.3. EMISSION FACTORS AND INVENTORIES

1.2.3.1. Existing Data

1. Regulation Packages
2. Open Literature

1.2.3.2. Models

1. Mobil—4
2. Part Ext

1.2.4. EMISSION CONTROLS—NOW AND PROJECTED

1.2.4.1. Engine Modifications

1. Combustion Chamber
2. Intake Port and Valves
3. Piston and Rings
4. Air Handling; Intercooling; Aftercooling
5. Electronic Control of Fuel Management

1.2.4.2. Add—on Devices—Description and Performance

1. Traps
2. Catalysts

1.2.4.3. Alternative Fuels — Performance

1. Natural Gas
2. Methanol
3. Reformed Diesel Fuel Oil

1.2.5. Conclusions

1.3. CONCENTRATIONS OF DIESEL-DERIVED POLLUTANTS IN AIR, THEIR TRANSPORT AND TRANSFORMATIONS

1.3.1. Fundamental Nature of Diesel Pollutants in Air

1.3.1.1. Gaseous pollutants

1.3.1.1.1. Inorganic gases

NO_x, CO, and SO_2 derived from diesel engines; relative contribution of diesel vehicles compared to gasoline-fueled vehicles.

1.3.1.1.2. Organic gases

Hydrocarbons and derivatives, PAH and derivatives, aldehydes, etc. Diesel compared to gasoline as a source of olefinic hydrocarbon in air.

1.3.1.2. Particulate phase pollutants

1.3.1.2.1. Vapor- and particle-phase distribution

Semi-volatile organic compounds (SOC), factors controlling vapor-to-particle ratio.

1.3.1.2.2. Particulate phase

PAH and derivates

PAH, nitroarenes, polar PAH.

1.3.1.2.3. Particulate carbon in air OC/EC, particulate size distribution, etc.

1.3.1.2.4. Other particulate phase pollutant sulfate and trace metals

1.3.2. Dispersion of Primary Emissions in Air

1.3.2.1. Aliphatic and aromatic hydrocarbons

1.3.2.2. Polycyclic Aromatic Hydrocarbons

1.3.2.3. Nitroarenes

1.3.3. Atmospheric Transformations in Air

1.3.3.1. Physical removal processes; wet and dry deposition, atmospheric transport

1.3.3.2. Nitroarene formation

1.3.3.3 Reactions on particles

1.3.4. Mutagenicity of ambient air particles Bioassay directed fractionation, etc.

1.4. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

1.4.1. Toxic Effects of Diesel Exhaust on Humans

Symptoms of acute exposure to high levels (i.e., above ambient) diesel exhaust include mucous membrane and eye irritation, headache, light-headedness, nausea, vomiting, heartburn, weakness, numbness and tingling in extremities, chest tightness, and wheezing. Exhaust odors can cause nausea, headache, and loss of appetite.

The effects of short-term exposures to diesel exhaust have been investigated primarily in occupationally exposed workers. In studies on underground miners, bus garage workers, dock workers, and locomotive repairmen, changes in respiratory symptoms and pulmonary function over the course of a workshift were generally found to be minimal and not statistically significant. However, in one study of bus garage workers, an increased frequency of symptoms of cough, labored breathing, itching, burning and watering of the eyes, chest tightness, and wheezing was observed. In addition, in some studies, reductions in forced vital capacity (FVC) and forced expiratory volume at 1 s (FEV_1) were measured in the exposed workers. The latter effects were attributed, in part, to nitrogen dioxide or to nitrogen dioxide adsorbed onto particulate matter.

Chronic effects of diesel exhaust exposure have been evaluated in epidemiological studies of occupationally exposed workers including miners, railroad yard workers, stevedores, and bus garage mechanics. In most cases the data have been insufficient to establish clear correlations between effects and exposure level or length of employment. Such correlations may be complicated by various factors including smoking habits, occurrence of allergies, frequency of virally induced respiratory problems, variations in exhaust composition and workplace ventilation, and the possible presence of other workplace air pollutants. In a few of these studies, a higher prevalence of respiratory symptoms, primarily cough and phlegm, were observed among the exposed workers. These symptoms were usually not accompanied by significant changes in pulmonary function; however, reductions in FVC and FEV_1, and occasionally in forced expiratory flow (FEF_{50}) and FEF_{75} have been reported. In one study, exposure to diesel exhaust was associated with chronic respiratory disease.

1.4.2. Toxic Effects of Diesel Exhaust on Animals

Animal studies on the toxic effects of diesel exhaust have involved acute, subchronic, and chronic exposure regimens. In acute exposure studies, toxic effects have been associated primarily with high concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. In short-term and chronic exposure studies, toxic effects have been associated with high concentrations of particulate matter. The data for short-term exposures (see Table 5-12) indicate minimal effects on pulmonary function even at exhaust concentrations sufficiently high to cause histological and cytological changes in the lungs. Exposures for several months or longer to levels far above ambient concentrations resulted in accumulation of particles in the lungs, increase in lung weight, increases in macrophages and leukocytes, macrophage aggregation, hyperplasia of alveolar epithelium, and thickening of the alveolar septa. Similar histological changes, as well as reductions in growth rates and alterations in parameters of pulmonary function, have been observed in chronic exposure studies. Several animal studies have also demonstrated reductions in resistance to respiratory tract infections in animals exposed to diesel exhaust. In addition, there are limited animal data associating minor behavioral and hematological changes, and cytological alterations in the liver with exposure to diesel exhaust.

In animals chronically exposed to diesel exhaust reductions in growth rates have been observed most often in studies in which the exhaust was diluted to produce concentrations of particulate matter of at least 2 mg/m^3 and in which the exposure periods lasted for 16 or more hours per day (see Table 5-13). In studies in which the daily exposures were only 6 to 8 h per day, no effects on growth were seen at particle levels of 6 to 8 mg/m^3.

Alterations in pulmonary function parameters that have been observed in animals chronically exposed to diesel exhaust include decreases in vital capacity, residual lung volume, diffusing capacity, dynamic lung compliance, and expiratory flow rates, as well as increases in airway resistance (Table 5-14). These effects were not reported in all exposure studies or for all species tested. They generally appeared only after prolonged exposures. The lowest exposure levels (expressed in terms of the concentration of exhaust particulate matter) that resulted in changes pulmonary function parameters occurred at 2 mg/m^3 in

cynomolgus monkeys, 11.7 mg/m^3 in cats, 1.5 and 3.47 mg/m^3 in rats, and 4.24 and 6 mg/m^3 in hamsters.

Typical histological findings in the lungs of animals chronically exposed to diesel exhaust include alveolar histiocytosis, macrophage aggregation, tissue inflammation, increase in polymorphonuclear leukocytes, hyperplasia of bronchiolar and alveolar Type II epithelial cells, thickened alveolar septa, edema, emphysema, and fibrosis (see Table 5-15). Lesions in the trachea and bronchi have also been observed in some studies. The exposure levels (exhaust particle concentrations) at which one or more of these effects occurred were 3.9, 4.24, and 6 mg/m^3 in hamsters, 1, 2, 2.2, 3.5, 4.24, 6.0, and 8.3 mg/m^3 in rats, 1.5 mg/m^3 in guinea pigs, and 4.24 mg/m^3 in mice. Associated with these histopathological findings are various histochemical changes in the lung, including increases in lung DNA, total protein, alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), increased synthesis of collagen, and release of inflammatory mediators such as leukotriene LTB and prostaglandin $PGF_{2\alpha}$.

Histopathological and histochemical changes appear to be dependent on the concentration of the exhaust particulate matter, and such changes have not been observed at low particle concentrations (i.e., 0.7 mg/m^3 in rats and 0.25 mg/m^3 in guinea pigs).

The histopathological changes caused by prolonged exposure to diesel exhaust appear to be strongly dependent on relative rates of pulmonary deposition and clearance. At particle concentrations of 1 mg/m^3 or above, pulmonary clearance becomes reduced and focal aggregations of particle-laden macrophages appear in the lungs, particularly in the alveolar and peribronchiolar regions, as well as in the hilar and mediastinal lymph nodes. With prolonged exposures, the continued presence of aggregations of soot-laden macrophages results in an inflammatory tissue response, septal thickening, epithelial proliferation, and fibrosis. The critical factor in this process is the minimal exposure, in terms of the concentration of the particulate matter, as well as in the length and frequency of the exposure periods, at which focal aggregations of soot-laden macrophages occurs. This exposure level varies from species to species and is likely to be dependent on rates of uptake, deposition, clearance, relative size of the macrophage population per unit of lung

tissue, rates of recruitment of macrophages and leukocytes, and relative efficiency of the mucociliary and lymphatic transport systems.

1.4.3. Interactive Effects of Diesel Exhaust Components

The physiological and toxicological effects of diesel emissions can be enhanced or altered as a result of the combined actions or interactions of the various exhaust components. Nitrogen dioxide, sulfuric acid, SO_2, and aliphatic aldehydes are chemical irritants, and one or more of these components may contribute to the eye and respiratory tract irritation observed in acute exposures to diesel exhaust. Several of these exhaust components, including NO_2, sulfuric acid, SO_2, and aliphatic aldehydes, are bronchoconstrictors. Sulfur dioxide and aliphatic aldehydes cause reductions in respiratory rates, while NO_2 may cause an increase. On an individual basis, any of these compounds could be expected to affect pulmonary function. However, the responses seen in short-term exposure studies with diesel exhaust are minimal, suggesting that concentrations are too low or that the methods used are not sensitive enough.

Impaired resistance to respiratory tract infections has been reported in some diesel exhaust studies. This may come about as a result of reduced mucociliary clearance, diminished effectiveness of alveolar macrophages, or reduced immunological competence. Nitrogen dioxide, acrolein, formaldehyde, and sulfuric acid may contribute to this effect as a result of their immediate cytotoxic activity and the resulting impairment of the mucociliary clearance mechanism in the respiratory tract.

The histological and cytological changes seen in the lungs of animals exposed to diesel exhaust have been attributed primarily to the accumulation and aggregation of exhaust particles in lung macrophages. Other exhaust components, particularly NO_2, aliphatic aldehydes, and sulfuric acid are also known to cause histopathological effects in the lungs. Most effects are directed at the bronchial and bronchiolar epithelial cells, with resulting hypertrophy and hyperplasia. Exposures to high levels of NO_2 can also lead to emphysematous lesions, which have also been reported in some diesel studies.

Adsorption of inorganic or organic compounds on diesel particulate matter may alter the chemical composition and toxicological effects of the exhaust. Sulfur dioxide and nitrogen dioxide can be adsorbed onto exhaust particles. The SO_2 could be catalytically converted to sulfuric acid. Attached to the exhaust particles, these substances may be carried deeper into lungs where they might have a more direct and potent effect on epithelial cells or on alveolar macrophages engulfing the particles. In addition, adsorption of these compounds may alter the acidity of the particles and thereby cause physicochemical changes affecting the polycyclic aromatic hydrocarbon fraction of the particles. Binding of the hydrocarbons and, consequently, bioavailablity may be altered by changes in pH, and this may result in more direct toxicological activity at the level of lung macrophages or lung epithelial surface.

1.4.4. Comparison of the Effects of Gasoline and Diesel Exhaust

Animal studies indicate that prolonged exposure to gasoline engine exhaust can result in histopathological changes in the lungs of exposed animals. These changes consist primarily of atypical bronchiolar epithelial hyperplasia causing, in some areas, partial occlusion of the bronchiolar lumina. Hyperplastic changes involving bronchiolar epithelial tissue also occur in chronic exposures to diesel exhaust. However, in the latter case there is usually extensive involvement of Type II cells in the alveolar region. This suggests that the mechanisms involved in the lung response to the two types of exhaust are similar but that the target tissue may be slightly altered due to the different physical and/or chemical composition of the exhausts. The higher levels of particulate matter in diesel exhaust may allow for a greater concentration of the active components of the exhaust in the deeper regions of the lungs. The effectiveness of diesel exhaust in inducing these tissue responses might also be enhanced by the presence of other exhaust components.

1.5. MUTAGENICITY OF DIESEL ENGINE EMISSIONS

Extensive studies with *Salmonella* have unequivocally demonstrated direct-acting mutagenic activity in both particulate and gaseous fractions of diesel exhaust. The induction of gene mutations has been reported in several *in vitro* mammalian cell lines after exposure to extracts of diesel particles.

Dilutions of whole diesel exhaust did not induce sex-linked recessive lethals in *Drosophila* or specific-locus mutations in male mouse germ cells. Structural chromosome aberrations and sister chromatid exchanges (SCE) in mammalian cells have been induced by particulates. Whole exhaust induced micronuclei, but not SCE or structural aberrations, in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 mo. In 7-week exposures, neither micronuclei nor structural aberrations were increased in bone marrow of female Swiss mice. Likewise whole diesel exhaust did not induce dominant lethals or heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

1.6. METABOLISM AND MECHANISM OF ACTION OF DIESEL EMISSIONS-INDUCED CARCINOGENICITY

Several studies affirm the bioavailability from inhaled diesel exhaust particles of compounds such as B[a]P and 1-nitropyrene (1-NP) which are known to be carcinogenic or mutagenic. Biotransformation of B[a]P, 1-NP, and some of the dinitropyrenes to reactive intermediates following inhalation of diesel exhaust particles has been verified. Furthermore, several reports have provided data indicating the formation of DNA adducts, considered an underlying mechanism of carcinogenicity, following administration of these compounds. The development of lung tumors in experimental laboratory animals following chronic exposures to particulate diesel exhaust occurs under conditions in which alveolar macrophage-mediated particle clearance from the lung is compromised. Although tumors have also been found to develop with other types of particles (e.g., titanium dioxide) when this clearance mechanism is diminished, tumors developing in the lungs of diesel emissions-exposed rats with less lung mass or comparatively less volume burden of diesel particles suggest that the

carcinogenic response is not exclusively related to an overabundance of particles in the lungs *per se*. Therefore, the organic components on diesel particles may be importantly involved in the development of lung tumors. The lung's pulmonary macrophages, which phagocytize deposited diesel particles, probably participate in the gradual *in situ* extraction and metabolism of procarcinogens associated with the diesel particles. Additionally, the normal tumoricidal activities of the pulmonary macrophages may be compromised upon interaction with excessive numbers of diesel particles, and diesel particle-macrophage interactions could lead to the generation of reactive oxygen species that have been shown to be at least mutagenic. Processes and potential mechanisms discussed herein have largely been derived from animal data, and further research is required to determine how the activities of human pulmonary macrophages in response to particulate diesel exhaust compare with pulmonary macrophages from experimental animals. Most importantly, valid dosimetry for the human condition will require the elucidation of the underlying mechanisms involved in the development of lung tumors following chronic exposure to whole diesel exhaust.

1.7. CARCINOGENICITY OF DIESEL ENGINE EMISSIONS IN LABORATORY ANIMALS

For human exposure considerations, inhalation is the most relevant mode of exposure. Only within the last 10 years have extensive, animal studies been conducted assessing chronic inhalation exposure of diesel exhaust. Studies employing rats and an adequate experimental design were nearly all positive in demonstrating diesel exhaust-induced increases in tumorigenicity. The 9.5 percent increase in tumor incidence for female Wistar rats exposed to whole diesel exhaust (4 mg/m^3) is supported by data showing a 3.6 percent and 12.8 percent tumor incidence in F344 rats following chronic exposure to diesel exhaust at particle concentrations of 3.5 mg/m^3 and 7.0 mg/m^3, respectively. Another study affirmed the observations of potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. Similarly, diesel exhaust-induced tumorigenicity was observed in rats exposed to an exhaust particle concentration of 4.9 mg/m^3, although the sample size was small. This study also reported development of a splenic lymphoma which represents the

only non-pulmonary tumor resulting from inhalation exposure to diesel exhaust. A long-term study showed a greater incidence of carcinomas (6.5 percent) in rats following 30 mo exposure to diesel exhaust at 4 mg/m^3 but not at lower (0.4, 1, or 2 mg/m^3) exposure levels. However, a dose-dependent increase in tumor incidence was reported for male and female F344 rats exposed to unfiltered diesel exhaust (five 16-h periods per week) at concentrations as low as 2.2 mg/m^3 and also at 6.6 mg/m^3. This study indicated that the tumor incidence was higher for female than for male rats. A more recent study reported a significant 6.5 percent increase in pulmonary carcinomas in male and female F344 rats chronically exposed to whole diesel exhaust (3.7 mg/m^3). Thus, these studies demonstrated carcinogenic effects in rats at exposure levels ranging from 2.2 to 7.0 mg/m^3. Additional studies also provided evidence of carcinogenicity in mice.

Two studies provided negative results for tumorigenicity of diesel exhaust in hamsters, a species known for its resistance to tumor induction. Negative results were also presented by several other investigators but these studies tended to employ inadequate exposure durations, low exposure concentrations, or inadequate animal numbers per group. Similarly, the studies using monkeys and cats were of inadequate duration (2 years) for these longer-lived species.

Alternate exposure routes including dermal exposure, skin painting, and subcutaneous injection provided additional evidence for tumorigenic effects of diesel exhaust. Evidence for tumorigenicity was observed for mice to which an acetone extract of diesel exhaust particles was applied dermally. Additionally, it has been shown that extracts from some diesel engines were tumorigenic following dermal application to rodents. A significant increase in the incidence of subcutaneous tumors in female C57BL mice was reported for subcutaneous administration of light-duty diesel exhaust tar extract at doses of 500 mg/kg. Additional data for this study identified an increased tumor incidence in the mice following injection of light-duty engine exhaust extract at doses of 100 and 500 mg/kg. Negative results were reported for skin painting studies using mice and acetone extracts of diesel exhaust particle suspensions, but particle collection procedures may have minimized the condensation of vapor-phase organics and, therefore, the availability of potentially carcinogenic components. Intraperitoneal injection studies were generally negative.

Experiments using tumor initiators such as DEN, B[a]P, DPIN, or DBahA did not provide conclusive results regarding the carcinogenic potential of filtered versus whole diesel exhaust. Some of these studies, however, strongly imply the importance of adsorbed organic compounds for the carcinogenic effect and data are available regarding the carcinogenic activity of several compounds known to be components of diesel exhaust.

Although the tumorigenicity of the gaseous fraction is presently unresolved and experiments using filtered exhaust were negative, most of these experiments did not provide definitive evidence that a maximum tolerated dose was achieved. It has not yet been unequivocally determined that the carbon core of the exhaust particle is not without carcinogenic potential.

Although uncertainties exist regarding the tumorigenic potential of the gaseous component and the carbon core component of diesel exhaust, it is clear that diesel exhaust is carcinogenic in animals. This contention is supported by positive results in numerous, independent studies, in male and females of at least two species, and by several routes of administration including, inhalation, intratracheal administration, skin painting, and subcutaneous injection.

1.8. PHARMACOKINETIC CONSIDERATIONS IN THE PULMONARY CARCINOGENICITY OF DIESEL ENGINE EMISSIONS

An understanding of the pharmacokinetics associated with pulmonary deposition of diesel exhaust particles and their adsorbed organics is critical in understanding the carcinogenic potential of diesel engine emissions. The pulmonary clearance of diesel exhaust particles is multiphasic and involves several processes including a relatively rapid mucociliary transport and slower macrophage-mediated processes. The observed dose-dependent increase in the particle burden of the lungs is due, in part, to an overloading of alveolar macrophage function. The resulting increase in particle retention has been shown to increase the bioavailability of particle adsorbed mutagenic and carcinogenic components such as B[a]P and 1-NP. Experimental data also indicate alveolar macrophage-mediated metabolism and phagolysosomal solubilization of particle-adsorbed components. Although

macromolecular binding of diesel exhaust particle-derived PAH and the formation of DNA adducts following exposure to diesel exhaust have been reported, a quantitative relationship between these and increased carcinogenicity is not available.

In addition to the aforementioned points, one must also consider the fact that other compounds (e.g gas-phase chemical irritants) may alter respiratory rate and, therefore the actual inhaled dose of potentially toxic components. Moreover, a better knowledge of particle dissolution rate and particle removal rate is necessary for more accurately assessing bioavailability of potentially carcinogenic components of diesel exhaust.

1.9. EPIDEMIOLOGY OF DIESEL EMISSIONS CARCINOGENICITY

A major difficulty with the epidemiological studies regarding exposure to diesel engine emissions was the measurement of the actual diesel exhaust exposure. Most studies compared persons in job categories with presumably some exposure to diesel exhaust to either standard populations (presumably no exposure to diesel exhaust) or with men in other job categories from industries with little or no potential for diesel exhaust exposure. A few studies have included measurements of diesel fumes, but there is no standard method for the measurement. Neither is any attempt made to correlate these exposures with the cancers observed in any of these studies nor is it clear exactly which extract should be measured to assess the occupational exposure to diesel exhaust. The occupations involving potential exposure to diesel exhaust are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators.

With the exception of one study, there have been no known studies of miners to assess whether diesel exhaust is associated with lung cancer. Virtually all miners (metal and coal) use diesel equipment, which was introduced in the early 1960s. Estimates of how many of these miners are exposed to diesel fumes are not available. The cohort studies mainly demonstrated an increase of lung cancer. Studies of bus company workers failed to demonstrate any statistically significant excess risk of lung cancer, but these studies have certain methodological problems such as small sample sizes, short follow-up periods, lack of

information on confounding variables and lack of analysis by duration of exposure or latency that preclude their use in determining the carcinogenicity of diesel exhaust.

A mortality study of heavy equipment operators demonstrated a significant increased risk of liver cancer in total and in various subcohorts. The same analysis also showed statistically significant deficits in cancers of the large intestine and rectum. Metastasis from the cancers of these sites in the liver probably got misclassified as primary liver cancer leading to observed excess risk of liver cancer. This study also found a non-significant positive trend for lung cancer with increasing length of membership and latency. However, due to flaws in methodology and data analysis, this study can not be used to support or refute a causal association between exposure to diesel exhaust and occurence of cancers.

After controlling for age and smoking, a two-year mortality analysis demonstrated an excess risk of lung cancer in certain occupations with potential exposure to diesel exhaust. These excesses were statistically significant among miners (RR = 2.67, 95 percent CI = 1.63 - 4.37) and heavy equipment operators (RR = 2.6, 95 percent CI = 1.12 - 6.06). The elevated risks were nonsignificant in railroad workers (RR = 1.59) and truck drivers (RR = 1.24). A dose response was also observed for the truck drivers. Despite the methodologic limitations such as the lack of representation of the study population comprised of volunteers only and the questionable reliability of exposure data based on self administered questionnaires which were not validated, this study is suggestive of a causal association between exposure to diesel exhaust and excess risk of lung cancer.

Two mortality studies were conducted on railroad workers, one in Canada and one in the United States. The Canadian study found relative risks of 1.2 (p <0.01) and 1.35 (p <0.001) among "possibly" and "probably" exposed groups, respectively. The trend test showed a highly significant dose response relationship with exposure to diesel exhaust and the risk of lung cancer. However, several limitations including lack of detail in work descriptions, confounding factors of simultaneous coal dust exposure, asbestos exposure, and smoking habits makes interpretation of this study difficult.

The U.S. study provided evidence for linking diesel exhaust exposure to lung cancer. Relative risks of 1.57 (95 percent CI = 1.19 to 2.06) and 1.34 (95 percent CI 1.02 to 1.76) were found for ages 40 to 44 and 45 to 49, respectively, after the exclusion of workers

exposed to asbestos. This study also found that risk of lung cancer increased with increasing duration of employment. This large cohort study, with lengthy follow up and adequate analysis including dose response (based on duration of employment as a surrogate) as well as adjustment for other confounding factors such as asbestos and smoking, make the observed association between increased lung cancer and exposure to diesel exhaust more meaningful.

Among the seven lung cancer case-control studies reviewed in this document, a study adjusting for age and smoking did not find an increased risk of lung cancer due to diesel fume exposure. A major limitation of this study was the lack of adequate exposure data derived from the job titles obtained from occupational histories. On the other hand, statistically non-significant excess risks were observed for diesel exhaust exposure in three different studies. The first study found the lung cancer excesses in railroad workers (OR = 1.4) and truck drivers (OR = 1.34). The second study found the excess for workers who were exposed to diesel exhaust versus who were not (OR = 1.4 and 1.7 with two different criteria), and the third study found the excess for professional drivers (OR = 1.2). These rates were adjusted for age and smoking. However, the first two studies had non participation rates of 47 percent and 36 percent respectively. In the second study the use of self reported exposures were not validated, which also had low power to detect excess risk of lung cancer for specific occupations.

After adjusting for smoking, significantly increased risks of lung cancer were found among French motor vehicle drivers (RR = 1.42) and transport equipment operators (RR =1.35). The main limitation of this study was inability to separate the exposures to diesel exhaust from that of gasoline exhausts since both motor vehicle drivers and transport equipment operators probably were exposed to the exhausts of both types of vehicles.

A group of investigators combined data from three studies (conducted in 3 different states) used to increase the power to detect an association of lung cancer with different occupations which had high potential for exposure to diesel exhaust. They found that truck drivers employed for more than 10 years had a significantly increased risk of lung cancer (OR = 1.5, 95 percent CI = 1.1 to 1.9). This study also found a significant trend of increasing risk of lung cancer with increasing duration of employment among truck drivers.

The relative odds were computed by adjusting for birth cohort, smoking, and state of residence. The main limitation of this study is again the mixed exposures to diesel and gasoline exhausts since information on type of engine was lacking. Potential bias may have been introduced because the ascertainment of cause of death for the selection of cases varied in three studies. The methods used to classify the occupational categories in these studies are different and, therefore, probably lead to incompatibility of occupational categories.

The most convincing evidence comes from the case-control study among U.S. railroad workers. After adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95 percent CI = 1.05 to 1.83). Among the younger workers with longer diesel exhaust exposure, the risk of lung cancer increased with the duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent diesel exhaust exposure (5 years before death), relative odds increased to 1.43 (95 percent CI = 1.06 to 1.94). This study appears to be a well conducted and well analyzed nested case-control study with reasonably good power. Potential confounders were controlled adequately and interactions between diesel exhaust and other lung cancer risk factors were tested.

Of the five bladder cancer case-control studies, three studies found increased risk in occupations with a high potential diesel exhaust exposure. A significantly increased risk of bladder cancer was found in Canadian railroad workers (RR = 9.0, 95 percent CI = 1.2 to 349.5) and in Argentinean truck and railroad drivers (RR = 4.31, $p < 0.002$). Significantly increased risks were observed with increasing duration of employment of ≥20 years in truck drivers (OR = 12, $p = 0.01$) and railroad industry workers (OR = 2.21, $p < 0.05$) in the third study. No significant increased risk was found for any diesel related occupations in the remaining two studies but both had several limitations such as inadequate characterization of diesel exhaust exposure, lack of validation of surrogate measures of exposure, presence of other confounding factors (urinary retention, concentrated smoke within the truck cab etc), most of them had small sample sizes, and none presented any latency analysis.

An excess risk of lung cancer was observed in three out of seven cohort studies and six out of seven case-control studies. Of these studies, two cohort and two case-control studies observed a dose response relationship using duration of employment as a surrogate

for dose. However, due to the lack of actual data on exposure to diesel exhaust in these studies and other methodologic limitations such as lack of latency analysis etc. the evidence of carcinogenicity in humans is considered to be limited for diesel exhaust exposure.

1.10. WEIGHT OF EVIDENCE CLASSIFICATION FOR CARCINOGENICITY AND QUANTITATIVE ESTIMATE OF UNIT RISK

On the basis of limited evidence for carcinogenicity of diesel engine emissions in humans, supported by adequate evidence in animals and positive mutagenicity data, diesel engine emissions are considered to best fit the weight-of-evidence category B1. Agents classified into this category are considered to be probable human carcinogens.

Two different approaches and several different equivalence dose assumptions are used to calculate unit risk estimates. In approach 1, deposition efficiency and a retardation of particle clearance from the alveolar region at high inhaled concentration, due to particle overload effect are taken into account by using mathmatical models. Approach 2 assumes that the lung burden is linearly proportional to the inhaled concentration in respective of the level of exposure. The approach that does not take into account the particle overload effect tends to predict higher risk when compared to those estimates where the overload effect is eliminated by either using the mathematical model or by using only data from low exposure groups in different studies (i.e., pooled data). When the particle overload effect is eliminated (or at least minimized when pooled data are used), the unit risk estimates are as follows: (Unit risk is equal to lifetime exposure to 1 $\mu g/m^3$)

- 3.0×10^{-6}, on organics per lung weight basis, by Approach 1*
- 1.3×10^{-5}, on organics per lung surface basis, by Approach 1*
- 1.0×10^{-6}, on organics per lung weight basis, pooled data, by Approach 1.
- 4.0×10^{-6}, on organics per lung surface basis, pooled data, by Approach 1.
- 1.0×10^{-6}, on body weight basis, pooled data, by Approach 2
- 2.3×10^{-5}, on air concentration basis, pooled data, by Approach 2
- 6.1×10^{-5}, on body surface basis, pooled data, by Approach 2

*Geometric mean unit risks from 3 studies

Of these unit risk estimates, the first two are considered to be the most reasonable because the effects of particle overload upon the dose of bioavailable, particle associated

organics are accounted for in the calculations. Although pooled data provide comparable risk estimates, it is questionable that pooling data from different studies is appropriate because these studies were not conducted under the same protocol, and secondly because the responses were quite small and variable. Of the first two estimates, the one based on organics per unit lung surface area ($1.3 \times 10^{-5}/\mu g/m^3$) is considered to be the most accurate because it most closely reflects concentration at the critical target organ. Uncertainties about these risk estimates and research needs to improve the dose-response modeling are elaborated in the discussion Section 7 Chapter 10.

1.11. REFERENCES

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NOTE: Chapter 2 is in preparation. However, an outline reflecting major topics is provided.

2. DIESEL EMISSIONS (OUTLINE)

The purpose of this chapter is to provide a basic understanding of how the Diesel engine contributes to the atmospheric cheical inventory attributed to mobile sources. Its five elements will provide information which will serve to put those chapters that follow in the proper context.

2.1. INTRODUCTION

2.1.1. Diesel Engine — What It Is and Why It Is Used.

1. Design Aspects — Two and four stroke cycle; naturally aspirated and turbocharged; fuel and lubricant systems
2. Typical Uses — Light-duty vehicles; line—haul trucks; buses; off—road equipment; locomotives

2.1.1. Fundamentals of Diesel Combustion — Heterogeneous with Excess Air.

1. Geometric Variables — Compression ratio; direct and indirect injection; injection timing
2. Operating Variables — Load; speed; lubricant; fuel

2.2. OVERVIEW OF POLLUTANTS AND POLLUTANT FORMATION

2.2.1. Gas Phase Emissions

1. Oxides of Nitrogen Formation
2. Hydrocarbon and Carbon Monoxide Formation

2.2.2. Carbon Formation and Emission

1. In Flames
2. Physical Characteristics of Emitted Particles

2.2.3. Gas—to—Particle Conversions

1. Organics————> Condensation — Absorption
2. Acids————> Oxidation — Condensation

2.2.4. Mutagens

1. Role of Nitrogen and its Oxides
2. Identification of Emitted Mutagens

2.3. EMISSION FACTORS AND INVENTORIES

2.3.1. Existing Data

1. Regulation Packages
2. Open Literature

2.3.2. Models

1. Mobil—4
2. Part Ext

2.4. EMISSION CONTROLS—NOW AND PROJECTED

2.4.1. Engine Modifications

1. Combustion Chamber
2. Intake Port and Valves
3. Piston and Rings
4. Air Handling; Intercooling; Aftercooling
5. Electronic Control of Fuel Management

2.4.2. Add—on Devices—Description and Performance

1. Traps
2. Catalysts

2.4.3. Alternative Fuels — Performance

1. Natural Gas
2. Methanol
3. Reformed Diesel Fuel Oil

2.5. Conclusions

NOTE: Chapter 3 has not been formatted for integration into the final document. The text headers, table numbers, and figure numbers are not consistent with the total document.

Chapter 3

DIESEL-DERIVED POLLUTANTS: ATMOSPHERIC CONCENTRATIONS, TRANSPORT, AND TRANSFORMATIONS

Draft Report

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1.0 INTRODUCTION

Combustion of fuel in an engine results in the formation of a complex mixture of gaseous and particulate exhaust. As discussed in detail in the previous chapter, the concentration of a chemical in the exhaust is a function of a number of factors, such as engine type, fuel composition, fuel and oil consumption, and engine operating conditions. During the late 1970s and early 1980s, because of a projected increasing usage of diesel engines in passenger cars and growing concerns over possible health effects associated with diesel particulate matter emissions, measurements were made to chemically characterize in detail the exhausts from light-duty and, to a lesser extent, heavy-duty diesel engines. Most of these measurements were of primary pollutants, that is, gases and particulate matter emitted directly into the air from their sources. In section 2 of this chapter, the available literature data concerning the chemical composition of primary diesel emissions and their distribution between gas- and particle-phase are reviewed.

However, it is important to realize that, once emitted, the primary pollutants are subjected to dispersion and transport and, at the same time, to chemical and physical transformations into secondary pollutants, which may be more harmful than their precursors. The time scales of these atmospheric transformations and physical loss processes vary widely, with atmospheric lifetimes ranging from < 1 min for some highly reactive organic compounds to months or even years for other much more inert constituents of direct emissions. Thus, to assess the impact of diesel emissions on human health and welfare, it is necessary to determine the chemical and physical changes that primary diesel emissions undergo during their transport through the atmosphere. Sections 3 and 4 of this chapter review the literature data concerning the concentration of

diesel-derived pollutants in ambient air and the atmospheric processes leading to their formation and disappearance.

2.0 PRIMARY DIESEL EMISSIONS

Diesel engine exhausts are complex mixtures containing thousands of chemical compounds in the particulate and gaseous phases. Sections 2.1 and 2.2 of this chapter review data concerning gaseous and particulate diesel engine emissions, respectively, and compare these data with those of spark-ignition engine emissions. Section 2.3 briefly reviews factors influencing emissions of certain organic compounds (mainly polycyclic aromatic hydrocarbons and their nitrated derivatives). The partitioning of the emitted organics between the gaseous and particulate phases is discussed in Section 2.4.

It has to be pointed out that the detailed chemical characterization of diesel engine emissions was performed mostly in the late 1970s and early 1980s. Since that time substantial changes have occurred in engine and emission control technologies and additional changes are to be expected in the future (see Chapter II). It is therefore reasonable to assume that the emissions from the currently manufactured diesel vehicles may not be the same as those measured and reported earlier. When possible, the latest data were used; however, the data presented in this chapter should not be considered as fully representative of either current emissions from the wide range of diesel engines presently used or of those that may have occurred in the past.

2.1 Gaseous Emissions

2.1.1 Inorganic Gases

The major products of the complete combustion of petroleum-based fuels in diesel and gasoline engines are carbon dioxide and water, with nitrogen from air constituting most of the remaining exhaust. A very small portion of the nitrogen is converted to nitrogen oxides and some nitrated hydrocarbons (IARC Monographs,

1989). Diesel exhausts also contain some sulfur dioxide, due to the presence of sulfur in the diesel fuel (on the average 0.2 wt %). Following combustion, approximately 98% of the sulfur is emitted as SO_2 and 2% as particulate sulfate (Pierson et al., 1978; Pierson et al., 1979; Truex et al., 1980). Most of the sulfate is in the form of H_2SO_4 (Truex et al., 1980).

Combustion of diesel fuel and incomplete combustion of gasoline results in the emission of CO and many gaseous- and particulate-phase organic compounds originating from unburnt fuel and lubricating oil. Table 2-1 shows the comparison of the average emission level of inorganic gases from light-duty diesel (LDD), heavy-duty diesel (HDD) and light-duty gasoline engines, with and without catalytic converter.

Table 2-1
Levels of Emissions from Current Diesel and Gasoline Engines (FTP cycle only)

Gases g/mile(g/km)	HDD	LDD	Gasoline Car	
			Noncat.	Catalyst
CO[a]	10 (6.25)	3 (1.9)	15 (9.4)	5 (3.1)
NOx[a]	28 (17.5)	1 (0.6)	4 (2.5)	2 (1.2)
SO_2[b]	1.6 (1.0)	.53 (.33)[c]	.1(.06)	.07(.05)

[a] From Schuetzle and Frazier (1986).
[b] From Pierson et al., 1979.
[c] From Truex et al., 1980.

The concentration of nitric acid (HNO_3) in light-duty diesel exhausts has been reported to be negligible in comparison to other anthropogenic sources (Okamoto et al., 1983; Harris et al., 1987). A range of concentrations from ~100 ppbv (~250 $\mu g\ m^{-3}$) to ~800 ppbv (~ 2 $mg\ m^{-3}$) [Harris et al., 1987] and an emission rate of ~ 1.3 mg/km were reported (Okamoto et al., 1983).

A small amount of ammonia was also detected in diesel engine exhausts (Pierson and Brachaczek, 1983b). The highest value of 25 mg/km of NH_3 was reported for heavy-duty diesels, ~ 4 mg/km for light-duty diesel and ~10 mg/km and ~5 mg/km for gasoline-powered vehicles, with and without catalyst, respectively (Pierson and Brachaczek, 1983b, and references therein). It was concluded that motor vehicles are an insignificant source of atmospheric NH_3.

2.1.2 Organic Gases

The emissions of gaseous organic compounds from diesel engines and spark-ignition engines are qualitatively similar (e.g., similar chemical components are present in both exhausts), although there are significant quantitative differences. Although the same federal emission standard of 0.41 g/mile applies to exhaust hydrocarbons for both diesel and spark-ignition light-duty vehicles, diesel engines emit more photochemically reactive hydrocarbons that are precursors of smog (National Research Council, 1982). In theory, new spark-ignition vehicles equipped with catalytic converters emit almost no reactive hydrocarbons in their exhausts. However, catalyst deterioration over the lifetime of the vehicle and the evaporative and refueling emissions cause an increase in the amount of reactive material released.

Table 2 shows the comparison of the emission rates of some representative alkanes, alkenes, aromatic hydrocarbons, and aldehydes from heavy- and light-duty diesel and gasoline engines, with and without a catalytic converter. Data on catalyst equipped gasoline vehicles are averaged from 46 in-use passenger cars, 1975-1982 models, selected to be representative of vehicles actually driven by

Table 2-2

Emission Rates of Volatile Organic Compounds (VOC) from Diesel and Gasoline Engines

VOC in g/mi (g/km)	HDD	LDD[a]	Gasoline Car	
			Noncat.[b]	Catalyst[c]
THC	-	.23(.14)[g]	5.4 (3.4)	1.8 (1.2)
Methane	-	.01(.008)	.27 (.17)	.26 (.16)
Ethylene	-	.04(.03)	.3 (.2)	.14 (.09)
Acetylene	-	-	.26 (.16)	.04 (.02)
Propylene	-	01(.008)	.15 (.09)	.04 (03)
n-Pentane	-	-	.09 (.06)	.03 (02)
iso-Pentane	-	-	27 (.17)	.07 (04)
n-Decane	01(.007)[d]	-	.003 (.0016)[e]	
n-Dodecane	.027(.017)[d]	-	.003 (.002)[e]	
Benzene	-	.02(.015)[f]	.31 (.19)	.06 (04)
Toluene	.01(.006)[f]	.006(.004)[g]	.7 (.45)	.1 (.07)
Xylenes	-	.002(.001)[g]	.96 (.6)	.08 (.05)
Ethyl benzene	.005(.003)[d]	.001(.0006)[g]	.21 (.13)	.02 (.01)
Naphthalene	-	.003(.002)[g]	-	-
Formaldehyde	-	.02(.01)	.06(.04)[f]	.025(.015)
Acetaldehyde	-	.007(.004)[g]	-	.01 (.007)
Acrolein	-	.01(.006)	-	.002(.001)
Benzaldehyde	-	-	-	.003(.002)
Total aldehyde	-	.03(.02)[g]	-	.04 (.03)

[a] From National Research Council (1982), except as indicated.
[b] From Bailey et al., 1990, except as indicated.
[c] From Sigsby et al., 1987.
[d] From Hampton et al., 1983, data from Allegheny Mountain Tunnel.
[e] As above, no differentiation between vehicles with and without catalyst.
[f] From Schuetzle and Frazier, 1986.
[g] From Smith, 1989, and Smith and Paskind, 1989; four-cycle FTP test, 1986 Mercedes Benz.

the U.S. public (Sigsby et al., 1987). Table 2 shows the data from the Federal Test Procedure (FTP) only (which attempts to simulate a typical urban driving pattern with average speed of ~ 20 mph), although two other driving cycles (Crowded Urban Expressway and the New York City cycle) are reported in the original publication.

The emission data on non-catalyst gasoline vehicles shown in Table 2 are averaged from 25 in-use passenger cars, representing late 1980s vehicle design in intensive use in the U.K. (Bailey et al., 1990). To allow direct comparison, the urban driving data (~ 13.5 mph average speed) are given in Table 2.

The data on light-duty diesel emissions are from the National Research Council Report (1982) and from the recently published (Smith, 1989; Smith and Paskind, 1989) studies concerning the evaluation of particle trap efficiencies for two light-duty diesel passenger cars, Mercedes Benz, 1986 model, and Volkswagen Jetta, prototype model. However, little has been published recently; most of the available data are from late 1970s and early 1980s and refer mainly to light-duty diesel. Quantitative data on emissions from heavy-duty vehicles are relatively sparse.

In addition, low concentrations of phenols were reported in heavy- and light-duty diesel emissions (Hare and Baines, 1979a; Hare and Baines, 1979b). Aliphatic carboxylic acids (mainly formic, acetic, propionic and benzoic acids) were also reported in vehicle exhausts (Kawamura et al., 1985), however no data exist concerning diesel engine emissions of these acids.

Table 2 lists the emission rates for exhaust pipe emissions only. Currently, fuel evaporation (e.g., from fuel lines and carburetors) accounts for 30 - 60% of the total hydrocarbon emissions from passenger gasoline vehicles with and without catalytic converter (IARC Monographs, 1989). The vapor pressure of

most current diesel fuels under ambient conditions is so low that emission due to evaporation of diesel fuels is not significant. However, for both diesel and gasoline engine emissions, methane, ethylene, and acethylene originate strictly from tail pipe emissions.

It has to be stressed that individual differences between vehicles of the same category in the quantity of emitted material are very significant and such differences arise from many factors of vehicle design, fuel control, engine conditions, and the general condition of the vehicle at the time of test. The differences between test parameters (e.g., speed, cold or hot start, fuel composition, etc.) make the comparison of the data given in Table 2 more uncertain. However, it is clear from these data, that the emission profile of gaseous organic compounds is different for diesel and spark-ignition vehicles; the aromatic hydrocarbons and low molecular weight alkanes ($< C_9$) are more characteristic of spark-ignition vehicle emissions, while the heavier alkanes ($>C_{10}$) are more characteristic of diesel emissions.

2.2 Particulate Emissions

2.2.1 Diesel Particulate Matter

The formation of particles is fundamental to the combustion process in diesel engines; it begins when fuel is injected into the combustion chamber and continues during and after the dilution of the exhaust into the atmosphere. As discussed in detail in the previous chapter, smaller primary spheres, formed during incomplete combustion of diesel fuel within the combustion cylinder, grow by agglomeration and by acting as nuclei for the condensation of organic compounds. The first steps of the process (injection, mixing, ignition, and combustion events) occur rapidly, within milliseconds, whereas the final steps,

taking place after dilution of the raw exhaust into the atmosphere, extend to hours or days.

Diesel particles are aggregates of spherical primary particles and 75-95% of the particulate mass is in the accumulation mode centered about an aerodynamic diameter of 0.2 μm. Figure 2-1 shows a typical size distribution of diesel exhaust particles (from National Research Council, 1982). The size distribution is important, because transport of the particles in the atmosphere (see Section 3.3) and deposition in the human respiratory tract depend essentially on aerodynamic diameter. Figure 2-2 shows the human respiratory system particle deposition efficiency curve (Heyder et al., 1986). The most important feature of this curve is the minimum in deposition in the range of 0.2 to 0.5 μm; particles of this size range are not retained effectively by the upper respiratory tract and can penetrate deep into the lung.

Diesel particulate matter is generally defined as any material that is collected on a filtering medium at a temperature 52 °C or less after dilution of the raw exhaust gases. Diesel engines produce 2 to 40 times and 2 to 10 times more particulate emissions than gasoline engines with and without catalytic converters, respectively (National Research Council, 1982). The main constituent of diesel particles is carbon, which accounts for ~ 80% of total particle mass. Approximately 70% of this so-called total carbon (TC) occurs in the form of soot or elemental carbon (EC); the rest is in the form of organic compounds and is called organic carbon (OC). Table 2-3 compares the emission rates of particulate matter and its distribution between total and organic carbon for heavy- and light-duty diesel and gasoline engines. Data on heavy- and light-duty diesel and

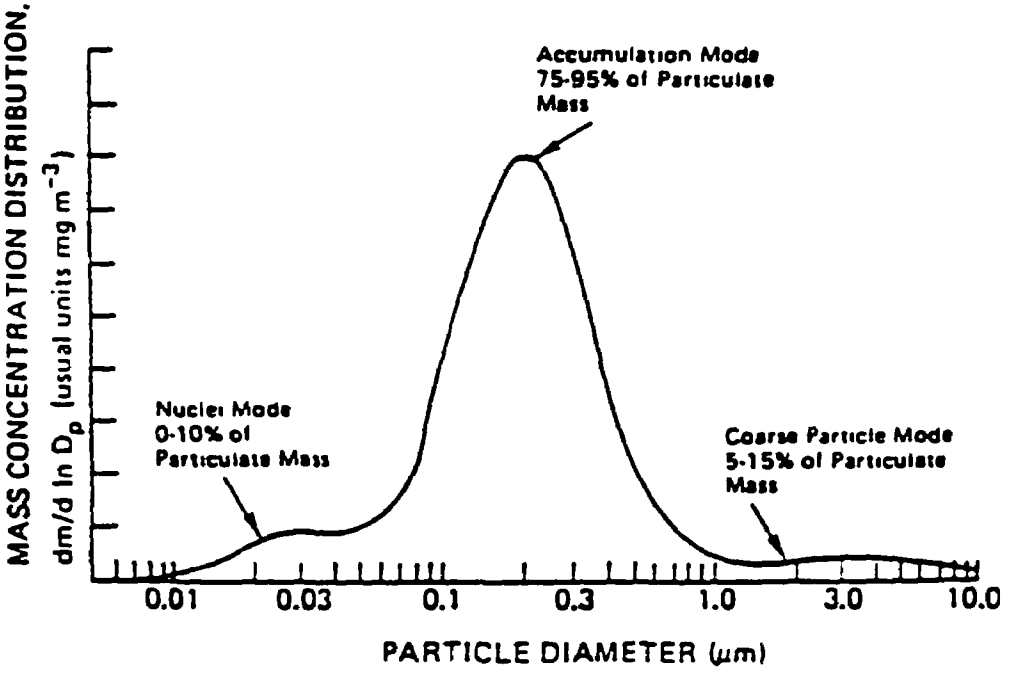

Figure 2-1 Typical size distribution of diesel exhaust particles (from National Research Council, 1982).

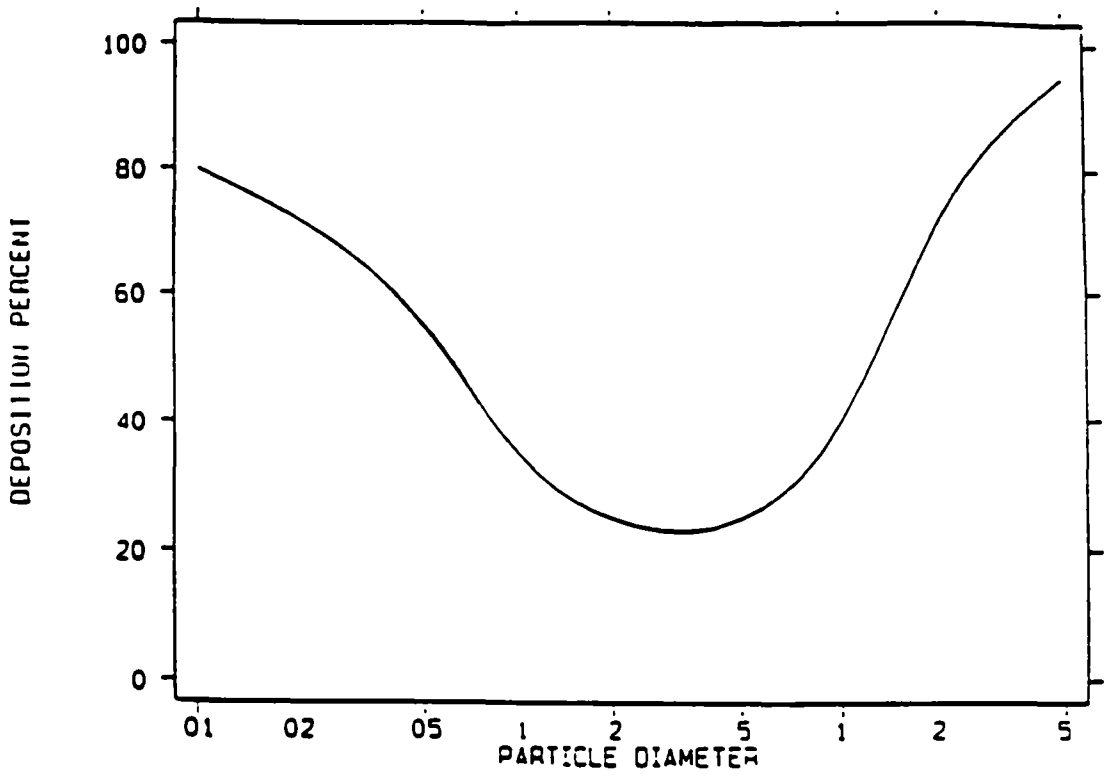

Figure 2-2 Example of human respiratory system aerosol particle deposition curve, adapted from Heyder et al., 1986. The dominant particle deposition mechanism on the left side of the curve is brownian diffusion; on the right side, it is impaction and sedimentation.

Table 2-3

Particulate Matter Emission Rates and Their Distribution Between Total Carbon (TC) and Organic Carbon (OC) for Heavy- and Light-Duty Diesel and Gasoline Engines

Emission	HDD	LDD	Noncat.	Catalyst
Particulate matter in g/mile (g/km)	3.2 (2)[a] 1.4 (.87)[d]	.6 (.37)[a] -	.1 (.07)[b]	.02 (.01)[c] .04 (.025)[d]
TC (% w/w)	78[a] 76[e]	80[a] -	31[b]	67[e]
OC (% of TC)	58[a] 30[d]	36[a]	87[b]	

[a] From Williams et al., 1989b.
[b] From Williams et al., 1989a.
[c] From Schuetzle and Frazier, 1986.
[d] From Pierson and Brachaczek, 1983a.
[e] From Szkarlat and Japar, 1983.

gasoline engines without catalytic converter are from the recently published survey of 13 HDD vehicles, 19 LDD vehicles, and 22 spark-ignition vehicles currently in intensive use in Sydney, Australia (Williams et al., 1989a; Williams et al., 1989b). The vehicles were tested on a dynamometer using several test procedures, but Table 2-1 lists the data from ADR 37 cycle, which is essentially identical to the 1979 U.S. FTP cycle.

Since the dynamometer studies are not fully representative of the conditions that exist on the road, the data obtained from the field experiments in two highway tunnels, the Allegheny and Tuscarora Mountain Tunnels of the Pennsylvania Turnpike (Pierson and Brachaczek, 1983a; Szkarlat and Japar, 1983), are also given in Table 2-3 for comparison.

2.2.2 Particulate Phase Inorganics

As discussed above, carbon, both organic and elemental, accounts for ~ 80% of total particulate matter mass. The remaining 20% is composed of sulfate (mainly H_2SO_4) [Pierson and Brachaczek, 1983a] and some inorganic additives and adventitious components of fuel and motor oil. Table 2-4 gives the average compositions of inorganic constituents of airborne particulate matter associated with vehicles on the road (from Pierson and Brachaczek, 1983a). All airborne constituents of particulate matter associated with vehicle traffic (other than atmospheric transformation products of primary emissions) are included, whether emitted from the exhaust or not (e.g., originated from tire wear debris and soil dust).

2.2.3 Particulate Phase Organic Compounds

Carbonaceous, diesel-emitted particles have high specific surface areas of 30 to 50 m^2/g (Frey and Corn, 1967). Due to this high surface area, diesel particles are capable of adsorbing relatively large quantities of organic material originating from unburned fuel and lubricating oil and from pyrosynthesis occurring during combustion of fuel (see Chapter II). After removal of extractable organic material, the surface area of diesel particles increases up to 90 m^2/g (Pierson and Brachaczek, 1976).

The extractable fraction of diesel particles is typically in the range of 20-30%, but it may be as high as 90% (Williams et al., 1989b) depending on vehicle type and operating conditions. In general, if a diesel engine is running under low load, the incomplete combustion leads to a relatively low particle concentration and a higher proportion of organic compounds associated with the particles (Dutcher et al., 1984).

Table 2-4

Summary of Composition and Emission Rates (mg/km) of Airborne Particulate Matter from On-Road Vehicles, Tuscarora Mountain Tunnel 1977 Experiment (from Pierson and Brachaczek, 1983a)

Constituent	Gasoline[a] mg/km (% of total mass)	Diesel[b] mg/km (% of total mass)
H	5 ± 4 (10 ± 6)	47 ± 11 (5 ± 1)
B	.04 ± .6 (.07 ± .11)	1.14 ± .16 (.13 ± .02)
C	34 ± 21 (67 ± 42)	725 ± 117 (84 ± 14)
N	1.1 ± .8 (2 ± 2)	16 ± 2 (1.9 ± .3)
Na[c]	.09 ± .37 (.2 ± .7)	6.6 ± 1.0 (.8 ± .1)
Mg[c]	7 ± .3 (1.3 ± .6)	8 ± 1 (.9 ± .15)
Al[c]	2 ± 5 (.3 ±.9)	8.5 ± 1 (1.0 ± .2)
Si[d]	.5 ± .7 (1.0 ± 1.3)	14 ± 2 (1.6 ± .2)
P[f]	07 ± .06 (.13 ±.11)	1.3 ± .2 (.15 ± .02)
S[SO_4=]	.4[3.4 ±.9] (.9[7±3])	23[42 ± 5] (2.7[4.9±.9])
Cl[e]	.8 ± .4 (1.6 ± .8)	0 (0)
K[d]	.17 ± .08 (.3 ± .2)	1.5 ± .2 (.17 ± .03)
Ca[c]	1.3 ± .3 (2.5 ± .7)	5.8 ± 1.4 (.7 ± .2)
Ti[d]	.006 ± .01 (.01 ± .02)	.12 ± .03 (.014 ± .004)
Mn[c,e]	.08 ± .01 (.16 ± .025)	.34 ± .04 (.04 ± .004)
Fe[c]	.32 ± .32 (.6 ± .6)	5.0 ± .9 (.6 ± .1)
Cu	.04 ± .02 (.07 ± .03)	.22 ± .09 (.025 ± .01)
Zn[f]	.04 ± .04 (.08 ± .08)	1.4 ± .1 (.16 ± 01)
Br[e]	5.75 ± .45 (11.2 ± .9)	0 (0)
Ba[e]	.03 ± .01 (.07 ± .02)	.66 ± .03 (.08 ± .033)
Pb[e]	12.4 ± 1.6 (24)	11.5 ± 3 (1.3 ± .3)

[a] Mostly passenger cars, no distinction between catalytic and noncatalytic vehicles.
[b] Mostly heavy-duty diesel trucks, average weight - 30 ton.
[c] Partially attributable to soil dust.
[d] Wholly attributable to soil dust.
[e] Attributable to fuel additives.
[f] Attributable to motor oil.

The extractable portion of total carbon, although commonly used as a measure of organic compound contents, is not totally equivalent to the organic carbon (OC) fraction as measured by the thermal-optical technique. The average ratio of organic carbon to extractable mass was shown to be 0.70 ± 0.05, when toluene/propanol-1 mixture was used as an extraction solvent (Japar et al., 1984), and this ratio was probably due to the presence of both oxygenated organic compounds and inorganic sulfates in the extracted mass.

2.2.3.1 Extraction and fractionation techniques. A variety of solvents and extraction techniques have been used in past for the separation of organic compounds from diesel particles (Levsen, 1988, and references therein). Although the reports on the extraction efficiencies are in part contradictory, it appears that Soxhlet extraction and the binary solvent system composed of aromatic solvent and alcohol gave the higher recovery of mass, as determined by ^{14}C-BaP [benzo(a)pyrene] spiking experiments (Schuetzle and Perez, 1981). Direct chemical analysis of the entire extractable fraction of diesel particulate matter is not generally possible because a large number of compounds of different polarity are present. The separation of diesel particulate organic matter (POM) into various fractions according to chemical functionalities is a necessary preliminary step to chemical identification of individual compounds. Open-column liquid chromatography (LC) and liquid-liquid separation procedures have been the most widely used fractionation methods (Lee and Schuetzle, 1983). Open-column LC is very often followed by normal-phase high performance liquid chromatography (HPLC), if the identification of less abundant components is required.

2.2.3.2 Chemical composition. Table 2-5 lists the general classes of organic compounds identified in particles from combustion emissions, including diesel emissions (from Schuetzle, 1988).

Table 2-5

Classes of Organic Compounds Identified in Particulate-Phase Combustion Emissions

Soluble Organic Material	Insoluble Organic Material
Hydrocarbons	Polymers
Derivatives[a] of hydrocarbons	Metal organic complexes
Polycyclic aromatic hydrocarbons (PAH)	Hydrocarbons ($>C_{40}$)
Derivatives[a] of PAH	
Multifunctional derivatives[a] of PAH	
Heterocyclic compounds	
Derivatives[a] of heterocyclics	
Multifunctional derivatives[a] of heterocyclic compounds	

[a] Derivatives include: acids, alcohols, aldehydes, esters, ketones, nitrates, sulfonates.

Liquid chromatography methods usually divide the complex environmental mixtures of organic compounds into nonpolar, moderately polar and polar fractions. This separation is achieved by the proper selection of solvents (or solvent mixtures) for the elution of compounds from chromatographic columns. Schuetzle and coworkers (1985) proposed that standard chemical compounds be selected for establishing reference points for the fractionation of diesel particulate organic matter (POM) into nonpolar, moderately polar, and polar fractions by normal phase HPLC. They proposed that the elution of 1-nitronaphthalene would define the end of the elution of nonpolar compounds and the beginning of the moderately polar fractions. In a similar way, 1,6-pyrene

quinone would define the end of elution of moderately polar compounds and the beginning of the polar region.

For light-duty diesel, ~ 57% of the extract mass is contained in the nonpolar fraction (Schuetzle, 1983). About 90% of this fraction consists of aliphatic hydrocarbons from approximately C_{14} to about C_{35} (Schuetzle, 1983), with a carbon number maximum at C_{22} to C_{26} (Black and High, 1979). Polycyclic aromatic hydrocarbons (PAH) and alkyl-substituted PAH account for the remainder of the nonpolar mass.

The moderately polar fraction (~ 9% w/w of extract) consists mainly of oxygenated PAH species and nitrated PAH. The polar fraction (~ 32% w/w of extract) is composed mainly of carboxylic and dicarboxylic acids of PAH, hydroxy-PAH, hydroxynitro-PAH, nitrated N-containing heterocyclic compounds, etc. (Schuetzle, 1983; Schuetzle et al., 1985).

Limited recovery studies have shown that there is little degradation or loss of diesel POM on the HPLC column. Greater than 90% of the mass and 70-100% of the Ames *Salmonella typhimurium*-active material injected onto the column have been recovered (Schuetzle et al., 1985).

2.2.3.3 Polycyclic aromatic hydrocarbon. Particle-bound PAH and their derivatives (mainly nitrated PAH) attracted considerable attention relatively early due to their mutagenic and, in some cases, carcinogenic properties (National Research Council, 1982). The most widely used methods of PAH analysis included thin layer chromatography (TLC), capillary gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and HPLC with UV or fluorescence detection (Levsen, 1988). Table 2-6 lists the PAH and thioarenes identified and

Table 2-6

Polycyclic Aromatic Hydrocarbons Identified and Quantified in Extracts of Diesel Particles (from Tong and Karasek, 1984)

Compound[a,b]	mol wt	Concentration (ng/mg extract)[c,d]
Acenaphthylene	152	30
Trimethylnaphthalene	170	140 - 200
Fluorene	166	100 - 168
Dimethylbiphenyl	182	30 - 91
C_4-Naphthalene	184	285 - 351
Trimethylbiphenyl	196	50
Dibenzothiophene	184	129 - 246
Phenanthrene	178	2,186 - 4,883
Anthracene	178	155 - 356
Methyldibenzothiophene	198	520 - 772
Methylphenanthrene	192	2,028 - 2,768
Methylanthracene	192	517 - 1,522
Ethylphenanthrene	206	388 - 464
4H-Cyclopenta(def)phenanthrene	190	517 - 1,033
Ethyldibenzothiophene	212	151 - 179
2-Phenylnaphthalene	204	650 - 1,336
Dimethyl(phenanthrene/anthracene)	206	1,298 - 2,354
Fluoranthene	202	3,399 - 7,321
Benzo(def)dibenzothiophene	208	254 - 333
Benzacenaphthylene	202	791 - 1,643
Pyrene	202	3,532 - 8,002
Ethylmethyl(phenanthrene/anthracene)	220	590 - 717
Methyl(fluoranthene/pyrene)	216	1,548 - 2,412
Benzo(a)fluorene/benzo(b)fluorene	216	541 - 990
Benzo(b)naphtho(2,1-d)thiophene	234	30 - 53
Cyclopentapyrene	226	869 - 1,671
Benzo(ghi)fluoranthene	226	217 - 418
Benzonaphthothiophene	234	30 - 126
Benz(a)anthracene	228	463 - 1,076
Chrysene or triphenylene	228	657 - 1,529
1,2-Binapthyl	254	30 - 50
Methylbenz(a)anthracene	242	30 - 50
3-Methylchrysene	242	50 - 192

Table 2-6 (continued)

Polycyclic Aromatic Hydrocarbons Identified and Quantified in Extracts of Diesel Particles (from Tong and Karasek, 1984)

Compound[a,b]	mol wt	Concentration (ng/mg extract)[c,d]
Phenyl(phenanthrene/anthracene	254	210 - 559
Benzo(j)fluoranthene	252	492 - 1,367
Benzo(b)fluoranthene	252	421 - 1,090
Benzo(k)fluoranthene	252	91 - 289
Benzo(e)pyrene	252	487 - 946
Benzo(a)pyrene	252	208 - 558
Benzo(ah)anthracene	278	50 - 96
Indeno(1,2,3-cd)pyrene	276	30 - 93
Benzo(ghi)perylene	276	443 - 1,050
Dibenzopyrene	302	136 - 254

[a] Compounds are arranged according to increasing GC retention times.
[b] Isomeric alkyl derivatives are not listed separately.
[c] Concentration range as found in the particulate extract of 3 VW passenger cars.
[d] Soluble organic fractions accounted for 11.1%, 12.1% and 14.7% of total particulate matter (w/w) for these three diesel samples.

quantified by GC/MS in three light-duty diesel particulate matter extracts (Tong and Karasek, 1984). Data listed in this table reveal the presence of a large number of alkyl derivatives of PAH, which are sometimes more abundant than the parent PAH.

Table 2-7 compares the emission rates of several representative PAH from heavy- and light-duty diesel and gasoline (with and without catalytic converter) engines.

Table 2-7

Emission Rates of Particle-Bound PAH from
Heavy- and Light-Duty Diesel and Gasoline Engines
(from Schuetzle and Frazier, 1986, except as indicated)

PAH in μg/mile (μg/km)	HDD	LDD	Gasoline Cars	
			Noncat.	Catalyst
Pyrene	1182 (739)	284 (177)	431 (269)	7 (4.4)
	-	39 (24)[a]	45 (28)[b]	-
		66 (42)[c]		
Fluoranthene	933 (583)	224 (140)	340 (212)	5 (3.1)
	-	50 (31)[c]	32 (20)[b]	-
Benzo(a)pyrene	54 (34)	13 (8.1)	20 (12.5)	4 (.25)
	-	1 (.6)[a]	3.2 (2)[b]	-
		ND[c]		
Benzo(e)pyrene	64 (40)	15 (9.4)	23 (14)	.4 (.25)
	-	3 (1.9)[a]	4.8 (3)[b]	-

[a] From Schuetzle and Frazier, 1986, but with 22% fuel aromacity.
[b] From Alsberg et al., 1985.
[c] From Smith, 1989, four-cycle FTP test, 1986 Mercedes Benz. ND - none detected.

2.2.3.4 Nitrated polycyclic aromatic hydrocarbon. Nitro-PAH (nitroarenes) have been shown to be present in diesel particulate extracts, however in much lower concentration than the parent PAH (Schuetzle et al., 1981; Schuetzle et al., 1982; Paputa-Peck et al., 1983). Since many nitroarenes are potent direct-acting (e.g., without metabolic activation) mutagens in the Ames assay using *Salmonella typhimurium* strains (Rosenkranz and Mermelstein, 1983, and references therein), the analysis of nitro-PAH in diesel particulate organic matter attracted considerable attention in the early 1980s.

A large number of nitro-PAH were identified in light-duty diesel particulate extracts using capillary GC with thermionic nitrogen-phosphorus (NPD) detector (Paputa-Peck et al., 1983). Positive isomer identification for 16 nitro-PAH has been made utilizing the GC retention times of authentic standards and low- and high-resolution mass spectra as identification criteria. These include 1-nitropyrene, 2-methyl-1-nitronaphthalene, 4-nitrobiphenyl, 2-nitrofluorene, 9-nitroanthracene, 9-methyl-10-nitroanthracene, 2-nitroanthracene, 2-nitrophenanthrene, 1-methyl-9-nitroanthracene, 1-methyl-3-nitropyrene, 1-methyl-6-nitropyrene, 1-methyl-8-nitropyrene, 1,3-, 1,6- and 1,8-dinitropyrene, and 6-nitrobenzo(a)pyrene. In addition two nitrated heterocyclic compounds were identified, namely 5- and 8-nitroquinoline. Forty-five additional nitro-PAH were tentatively identified in this diesel particulate extract (Paputa-Peck et al., 1983).

The concentration of nitro-PAH adsorbed on diesel particles varies substantially from sample to sample. Usually 1-nitropyrene is the predominant component, and concentrations ranging from ~7 to ~165 μg/g of particles are reported (Levsen, 1988).

Table 2-8 gives the approximate concentrations of several more abundant nitro-PAH in light-duty diesel particulate extracts (from Campbell and Lee, 1984, unless noted otherwise).

2.2.3.5 Oxygenated PAH. The moderately polar fraction of diesel particulate extract contains a variety of oxy-PAH (in particular aldehydes, ketones, quinones and acid anhydrides) in much higher amounts than nitro-PAH, which elute in the same fraction. Oxy-PAH are nonmutagenic or very weakly mutagenic, which explains the relatively low interest in this group of compounds. The most detailed study of oxy-PAH was published by Schuetzle et al. (1981), who identified more than 100 compounds. A large number of oxy-PAH in the molecular weight range of 182-272 were also identified by Tong et al. (1984). The main components identified by Tong and coworkers in particulate matter extracts of three light-duty diesel cars (VW) were: 9-fluorenone, anthraquinone, 4H- cyclopenta(def)phenanthrene-4-one, 9-phenanthrene aldehyde, benzo(de)anthracene-7-one, and benzo(cd)pyrene-6-one. These components are present at concentrations of 30-300 $\mu g/g$ particles. Some of these oxy-PAH are formed during sampling (Levsen, 1988).

2.2.3.6 Polar PAH derivatives. While 65-75% of the directly-acting mutagenicity (as tested by Ames *Salmonella typhimurium* assay) for light-duty diesel particulate extracts is associated with the fraction of moderate polarity, more than 65% of the mutagenic activity for heavy-duty diesel particulate extract is concentrated in the most polar fraction (Schuetzle et al., 1985). However, due to the serious analytical difficulties, only preliminary data exist on the identification of compounds which are responsible for this mutagenic activity (so

Table 2-8

Concentrations of Nitro-PAH Identified in a LDD Particulate Extract

Nitro-PAH[a]	Concentration (μg/g of particles)
4-nitrobiphenyl	2.2
2-nitrofluorene	1.8
2-nitroanthracene	4.4
9-nitroanthracene	1.2
9-nitrophenanthrene	1.0
3-nitrophenanthrene	4.1
2-methyl-1-nitroanthracene	8.3
1-nitrofluoranthene	1.8
7-nitrofluoranthene	0.7
3-nitrofluoranthene	4.4
8-nitrofluoranthene	0.8
1-nitropyrene	18.9; 75[b]
6-nitrobenzo(a)pyrene	2.5
1,3-dinitropyrene[b]	0.30
1,6-dinitropyrene[b]	0.40
1,8-dinitropyrene[b]	0.53
2,7-dinitrofluorene[c]	4.2; 6.0
2,7-dinitro-9-fluorenone[c]	8.6; 3.0

[a] From Campbell and Lee, 1984, unless noted otherwise. Concentrations recalculated from μg/g of extract to μg/g of particles using a value of 44% for extractable material (w/w)

[b] From Paputa-Peck et al., 1983.

[c] From Schuetzle, 1983.

called "polar mutagens"). Schuetzle and co-workers (Schuetzle et al., 1985) employed the concept of "bioassay directed chemical analysis" (see Section 5 of this chapter) for the isolation and identification of polar PAH derivatives from the extracts of heavy-duty particulate matter (NIST standard reference material, SRM 1650). Several hydroxynitro-PAH, hydroxy-PAH, and nitrated heterocyclic compounds were tentatively identified in the polar fraction.

In another study (Bayona et al., 1988), the polar HPLC fractions of the same NIST SRM 1650 were analyzed by fused silica capillary GC with low- and high-resolution mass spectrometry (MS), using electron impact (EI) and negative ion chemical ionization (NICI) techniques In addition, direct-probe EI and NICI-MS analyses were performed. Over 80 polycyclic aromatic compounds (PAC) belonging to several different chemical classes (anhydrides, carboxaldehydes, diazaarenes, cyclic imides, hydroxynitro-PAH, nitroaza-PAC, nitrolactones, and quinones) were tentatively identified. Ten of them were positively identified by comparison of retention times with authentic standards. Among them, phenazine and phthalic anhydride were positively identified for the first time in diesel exhaust particles. In addition, cyclic imides and their alkylated derivatives were tentatively identified.

2.3. Factors Influencing Emissions of PAH and Nitro-PAH

The influence of diesel engine type and make, diesel fuel aromaticity, and engine operating conditions (engine timing and load) on the emission level of pyrene, benzo(a)pyrene, benzo(e)pyrene, and 1-nitropyrene and mutagenicity (*Salmonella typhimurium* strain TA98 with and without metabolic activation) of diesel particulate extracts has been investigated (Schuetzle and Frazier, 1986;

Schuetzle and Perez, 1983). Table 2-9 summarizes the results (from IARC Monographs, 1989) of these experiments.

Particulate samples were collected from four light-duty diesel vehicles produced by four major manufacturers. The emission rates of the four test compounds varied by no more than factor of three (see Table 2-9). Also, the differences in mutagenicities of the total exhaust particulate extracts were not very significant.

Table 2-9 also shows that the emission of PAH and total mutagenicity increased by a factor of 3-4 when the fuel aromaticity was increased from 22% to 55% The 22% and 55% fuels contained 2-24 and 2-60 mg/l of pyrene, respectively. The amount of pyrene in the particulate emissions was not related to the pyrene content in the fuel, indicating that PAH are formed primarily as a result of the combustion process and are not due to the unburned fuel in the exhaust. In addition, fuel aromaticity had no effect on the 1-nitropyrene emission. These facts suggested that the presence of NO_x species and not pyrene content is the limiting factor in the chemical formation of 1-nitropyrene.

Changes in engine timing (see Table 2-9) had little effect on the PAH emissions but both 1-nitropyrene and mutagenicity increased by several fold as the timing was advanced, correlating with the increase in emissions of nitrogen oxides. Engine speed and load had the most significant effect on the emission of PAH and 1-nitropyrene (Schuetzle and Perez, 1983). High load and speed raise engine and exhaust temperatures enhancing partial oxidation of PAH and nitro-PAH, thereby resulting in reduction of PAH and nitro-PAH emissions relative to the partially oxidized PAH derivatives.

Table 2-9

Factors Affecting Rate of Emission of Polycyclic Aromatic Hydrocarbons in µg/mile (µg/km) from Diesel Engine Exhaust and Mutagenicity[a]

	Vehicle[b]				Fuel aromaticity[c]		Engine conditions[d]		
	A	B	C	D	22%	55%	Retarded timing	Standard timing	Advanced timing
Pyrene	39±16	62±15	29±15	24±11	39±18	125±39	31±20	39±18	35±22
	(24±10)	(39±9)	(18±9)	(15±7)	(24±11)	(78±24)	(19±13)	(24±11)	(22±14)
Benzo[a]pyrene	1 3±0 9	1 9±0 1	1 6±0 2	0 6±0 1	1 3±0 5	7 1±3 6	1 7±1 1	1 3±0 5	1 5±0.6
	(0 8±0 6)	(1 2±0 06)	(1±0 1)	(0 4±0 06)	(0 8±0 3)	(4 4±2 3)	(1 1±0 7)	(0 8±0 3)	(0 9±0 4)
Benzo[e]pyrene	2 3±1.2	5 1±0 2	3 0±0 4	1 3±0 4	3 0±1 1	10 3±4 1	3 6±2 1	3 0±1 1	4.2±0 5
	(1 4±0.8)	(3±0.1)	(1 9±0 3)	(0 8±0 3)	(1 9±0 7)	(6 4±2 6)	(2 3±1 3)	(1 9±0.7)	(3±0 3)
1-Nitropyrene	3 0±1.0	7 8±2 2	1 8±0 9	3 8±1 4	4 1±1 9	3 7±1 6	2 3±0 5	4 1±1 9	15 5±7 7
	(1 9±0.6)	(4 9±1 4)	(1 1±0 6)	(2.4±0 9)	(2 6±1 2)	(2 3±1)	(1.4±0 3)	(2 6±1 2)	(10±5)
Nitrogen oxides in g/mile (g/km)	-	-	-	-	-	-	0 9±0 02	1 0±0 01	1 3±0 1
							(0 6±0 01)	(0 6±0 006)	(0 8±0 06)
Mutagenicity in 10^6 rev/mile (10^6 rev/km)									
TA98(without activation)	1 0±0 4	1 3±0 3	0 8±0 3	0 8±0 2	0 99±0 35	2 9±0 80	2 2±1 6	3 4±1 5	6 4±2 7
	(0 6±0 2)	(0 8±0 2)	(0 5±0 2)	(0 5±0 1)	(0 6±0 2)	(1 8±0 5)	(1.4±1.0)	(2 1±0 9)	(4.0±1.7)
TA98 (with activation)	0 5±0 2	0 7±0 2	0 6±0 2	0 5±0 1	0 61±0 18	2 1±0 49	1 0±0 5	1 8±0 7	2.5±1 1
	(0.3±0.1)	(0.4±0 1)	(0 4±0 1)	(0 3±0 06)	(0 4±0 1)	(1 3±0 3)	(0.6±0 3)	(1 1±0 4)	(1.6±0.6)

[a] From IARC Monographs (1989), based on data given by Schuetzle and Frazier (1986)

[b] Duplicate tests for each vehicle (Columns A, B, C, D) run on four different fuels of 22% aromatic composition

[c] Duplicate tests on four vehicles run at standard timing on four different fuels

[d] Duplicate tests on two vehicles.

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2.4 Gaseous/Particulate Phase Emission Partitioning

The distribution of the emissions between the gaseous and particulate phases is determined by the vapor pressure of the individual species, by the amount and type of the particulate matter present (adsorption surface available), and by the temperature (Ligocki and Pankow, 1989). Table 2-10 gives the vapor pressures at 25 °C of some representative PAH ranging from naphthalene to benzo(a)pyrene.

The factor of $\sim 10^7$ in the range of vapor pressures is reflected in the fact that, at equilibrium at ambient temperature, naphthalene exists almost entirely in the gas phase, while BaP, other five-ring, and higher-ring PAH are predominantly adsorbed on particles. The intermediate three- and four-ring PAH are distributed between two phases.

However, the vapor pressures of these intermediate PAH can be significantly reduced by their adsorption on various type of surfaces. Because of this phenomenon, the amount and type of particulate matter present plays an important role, together with temperature, in the vapor-particle partitioning of semivolatile organic compounds (SOC).

The measurements of gas/particulate phase distribution are often accomplished by using a high-volume filter followed by an adsorbent such as polyurethane foam (PUF), Tenax, or XAD-2 (Cautreels and Van Cauwenberghe, 1978; Thrane and Mikalsen, 1981; Yamasaki et al., 1982). However, the pressure drop behind a hi-vol filter or cascade impactor contributes to volatilization of the three- to five-ring PAH, to a degree reflecting their vapor pressures. The magnitude of this "blow-off" artifact depends on a number of factors including sampling temperature and the volume of air sampled (Van Vaeck et al., 1984; Coutant et al., 1988). Despite these problems from volatilization, measurements

Table 2-10

Vapor Pressures at 25°C
for a Series of PAH[a]

PAH	Vapor Pressure at 298 K (torr)
Naphthalene	8.0×10^{-2}
Acenaphthylene	6.7×10^{-3}
Acenaphthene	2.2×10^{-3}
Fluorene	6.0×10^{-4}
Phenanthrene	1.2×10^{-4}
Anthracene	6.0×10^{-6}
Fluoranthrene	9.2×10^{-6}
Pyrene	4.5×10^{-6}
Benzo[a]anthracene	2.1×10^{-7}
Benzo[a]pyrene	5.6×10^{-9}
Chrysene[b]	6.4×10^{-9}

[a]Sonnefeld et al., 1983, except as indicated.
[b]Yamasaki et al., 1984.

with the hi-vol filters followed by a solid adsorbent have provided most estimates of vapor-particle partitioning of SOC in ambient air, as well as insights into the factors influencing SOC adsorption onto aerosols.

Average distributions of PAH between hi-vol filter and PUF plugs (positioned downstream of the filter) in samples collected in a heavily traveled roadway tunnel (Baltimore Harbor Tunnel), are shown in Figure 2-3 (from Benner et al., 1989). As discussed above, the "blow-off" from the filter preclude detailed quantitative interpretation. However, it can be seen from this figure that significant fractions of phenanthrene, anthracene, and their alkylated derivatives, along with fluoranthene and pyrene, exist in the gas phase. No PAH less volatile than pyrene were observed in any of the PUF samples. Comparison of the observed vapor-to-particle PAH ratios and those calculated based on the relationship derived by Yamasaki and co-workers (1982), generally agreed within a factor of two (Benner et al., 1989).

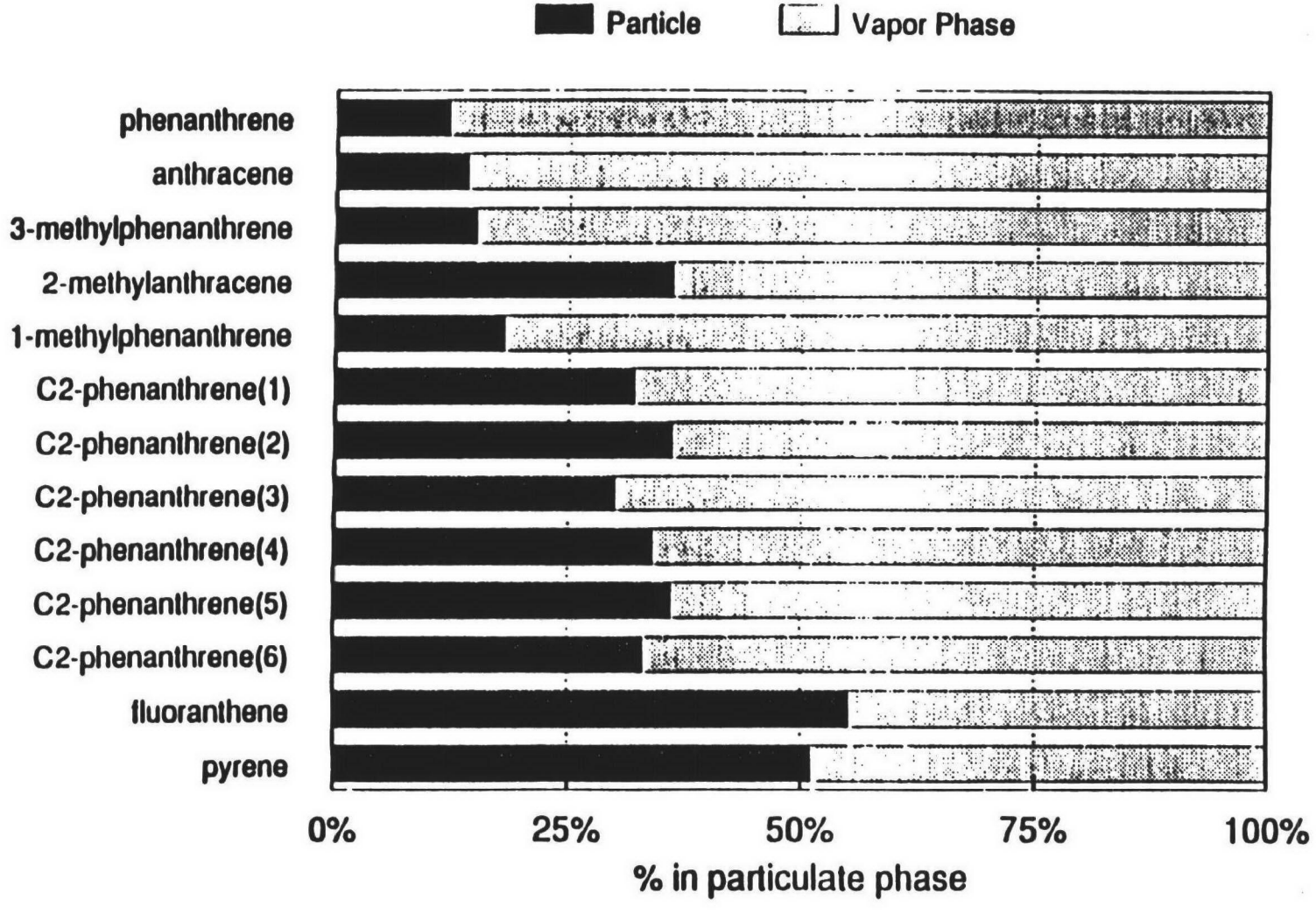

Figure 2-3. Vapor/particle phase PAH distribution in samples collected in Baltimore Harbor Tunnel

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3.0 ATMOSPHERIC TRANSFORMATIONS OF PRIMARY DIESEL EMISSIONS

3.1 The Fate of Primary Diesel Emissions and the Long Range Transport

Once released into the atmosphere, primary diesel emissions (or any other direct emissions) are subjected to dispersion and transport and, at the same time, to various physical and chemical processes that determine their ultimate environmental fate. The role of the atmosphere may be compared in some way to that of a giant chemical reactor in which materials of varying reactivity are mixed together, subjected to chemical and/or physical processes and finally removed (Schroeder and Lane, 1988) The main features of the atmospheric cycle for primary diesel emissions, beginning with emission and ending with deposition to the Earth's surface, are shown on Figure 3-1 (from Schroeder and Lane, 1988).

Initial mixing describes the physical processes that act on pollutants immediately after their release from an emission source. The dilution of diesel exhaust under roadway conditions is an important factor to consider; whereas a dilution factor of 10 is typical of many dilution tunnels used in dynamometer studies of automobile exhaust, a dilution factor of $\sim 10^3$ is more realistic under roadway conditions. This discrepancy may lead to slightly different particle size distributions under real driving conditions than those predicted from laboratory data (Kittelson and Dolan, 1980); e.g., due to slower coagulation processes, more particles in the Aitken nuclei range (≤ 0.08 μm diameter) may be expected under typical roadway conditions.

Diffusion and transport processes occur simultaneously in the atmosphere and account for the dispersion of emissions. The actual distance travelled by gaseous- and particulate-phase pollutants depends on the amount of time a specific pollutant resides in the atmosphere and is available for dispersion (Schroeder and Lane, 1988). As was discussed in the previous section, primary

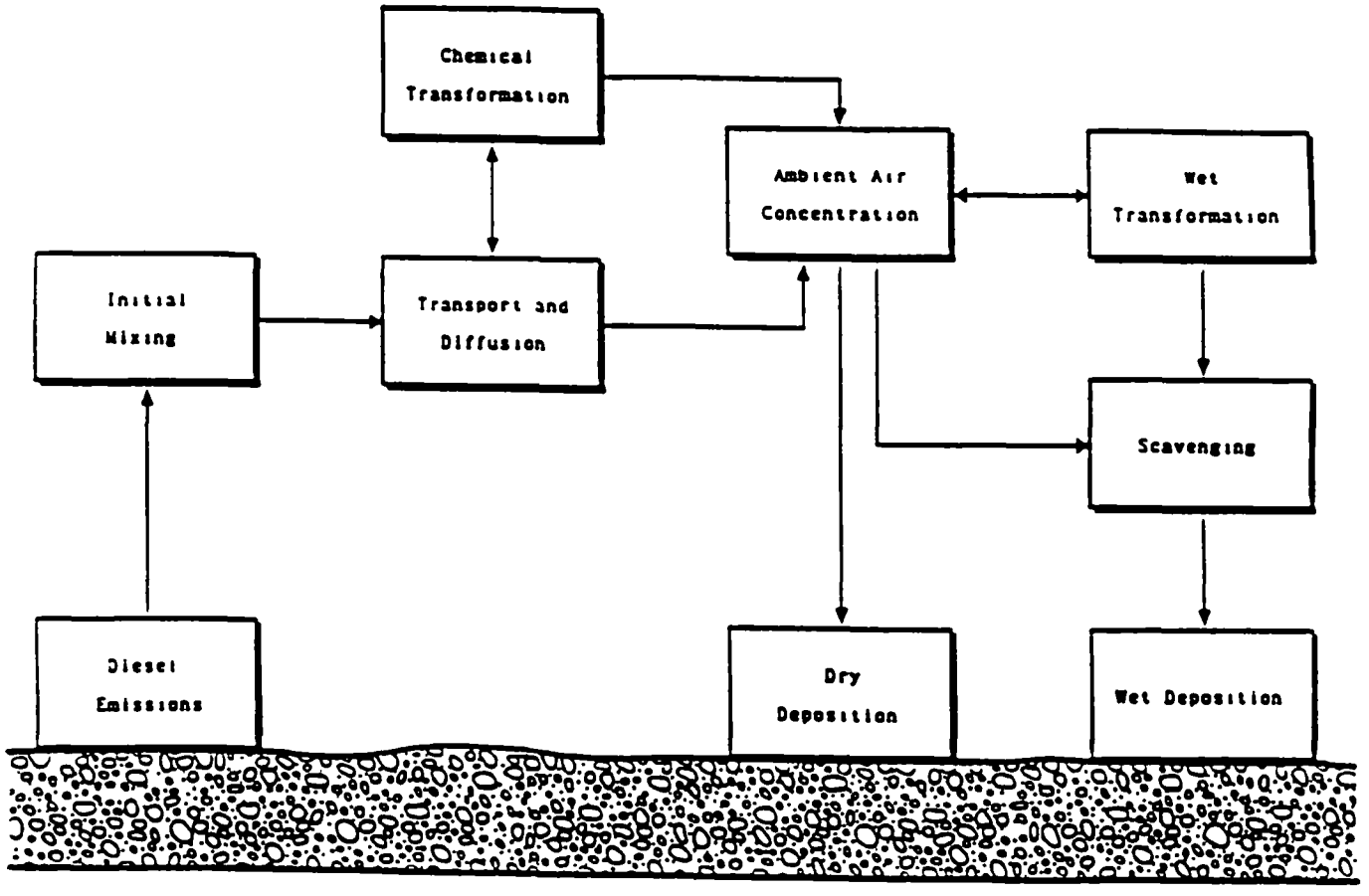


Figure 3-1. Diesel-derived pollutants: emission-to-deposition atmospheric cycle.

diesel emissions are a very complex mixture containing thousands of organic and inorganic constituents in the gas and particulate phases. These compounds have different chemical reactivities and are removed by dry and wet deposition processes with different rates as discussed more thoroughly in Sections 3.2 and 3.3. As a result, more reactive compounds with short lifetimes will be removed from the atmosphere relatively quickly, whereas more stable pollutants can be transported over greater distances. Clearly, a knowledge of the atmospheric loss processes and lifetimes for automotive emissions is important, since these lifetimes determine the geographic extent of the influence of these emissions.

It is now well established that man-made pollutants can travel through the atmosphere over long distances. In particular the long-range transport of SO_2 and its transformation to $SO_4^=$ have been studied extensively (see, for example, Galloway and Whelpdale, 1980; Lowenthal and Rahn, 1985). The organic pollutants, particularly those adsorbed on carbonaceous particles, are also subjected to long-range transport. As will be discussed in Section 3.2.2, organic compounds, such as polycyclic aromatic hydrocarbons (PAH), adsorbed on diesel particulate matter are generally more resistant to atmospheric reactions than those in the gas phase. In addition, particles of smaller diameter (< 1 μm), such as diesel particulate matter (see Figure 2-1, Section 2.2.1) are removed less efficiently by wet and dry deposition (see Section 3.3), thus having longer atmospheric residence times.

It has been reported (Laflamme and Hites, 1978; Hites et al., 1980) that PAH and their alkyl homologs are distributed in sediments throughout the world and that the PAH patterns are similar to each other and to air particulate matter for most of the locations studied. Furthermore, the quantities of PAH increase

with proximity to urban areas. This suggests anthropogenic combustion sources and long-range atmospheric transport of PAH.

Conclusive evidence for long-range transport of PAH was also reported from the recent measurements of PAH in Siskiwit Lake, located on a wilderness island in northern Lake Superior (McVetty and Hites, 1988). Because of its remote location, any PAH found in this lake must have originated exclusively from atmospheric transport.

Earlier studies by Bjørseth and coworkers (Bjørseth and Olufsen, 1983, and references therein) showed that PAH are transported from Great Britain and from the European continent to remote locations in Norway and Sweden. The specific sources of PAH emissions could not be identified, however. The authors speculated that combustion engines were not the major sources of PAH, since the amounts of BaP found in the samples collected in Norway and Sweden were higher than could be accounted for from gasoline and diesel fuel consumption in Great Britain and since the coronene/BaP ratios in the samples were lower than usually found in gasoline and diesel exhaust. However, due to the lack of PAH profiles specifically for combustion engines (or, as a matter of fact, any other specific tracer) the problem of relative contribution of gasoline and diesel vehicle exhausts to long-range transport of organics cannot be solved at the present time. The need for such "organic profiles" is addressed in Section 4.4 of this Chapter.

3.2 Chemical Transformations

3.2.1 Gas-phase Reactions

For gas-phase compounds, the following chemical processes contribute to their removal from the atmosphere (Atkinson, 1988):

- Photolysis during daylight hours.
- Reaction with hydroxyl (OH) radicals during daylight hours.
- Reaction with ozone (O_3) during daytime and nighttime.
- Reaction with hydroperoxyl (HO_2) radicals during, typically, late daytime and early nighttime hours.
- Reaction with gaseous nitrate (NO_3) radicals during nighttime hours.
- Reaction with dinitrogen pentoxide (N_2O_5) during nighttime hours[1].
- Reaction with NO_2 during daytime and nighttime hours.
- Reaction with gaseous nitric acid (HNO_3) and other species such as nitrous (HNO_2) acid and sulfuric acid (HSO_4).

The reactive gaseous species, such as OH radicals, NO_3 radicals, HO_2 radicals, and ozone, are present in the atmosphere either during the daytime (OH radicals) or nighttime (N_2O_5 and NO_3 radicals) hours or both time periods (ozone, NO_2). For the routes of formations of these species and their concentrations in the troposphere, see Finlayson-Pitts and Pitts, 1986.

Table 3-1 gives the calculated atmospheric lifetimes for some selected compounds present in automotive gas-phase emissions due to known tropospheric chemical removal reactions (from Atkinson, 1988). These lifetimes (i.e., the time for the compound to decay to 1/e or 37% percent of its original concentration) are calculated from the corresponding measured reaction rate constants and the average ambient concentration of the tropospheric species involved.

---

[1]It has been shown recently (Atkinson et al., 1990) that the N_2O_5 reactions with polycyclic aromatic hydrocarbons proceed by initial NO_3 addition to form an NO_3-PAH adduct which either dissociates back to reactants or reacts exclusively with NO_2 to form nitroarenes and other products. Since under atmospheric conditions, where N_2O_5, NO_3 radicals, and NO_2 are in equilibrium, these reactions are kinetically equivalent to a reaction with N_2O_5 with an effective N_2O_5 reaction rate constant, we will further refer to these reactions as N_2O_5 reactions.

Table 3-1

Calculated Atmospheric Lifetimes for Gas-Phase Reactions of Selected Compounds Present in Automotive Emissions with Atmospherically Important Reactive Species (from Atkinson, 1988, unless noted otherwise)

Compound	Atmospheric Lifetime Due to Reaction with:				
	OH[a]	O_3[b]	NO_3[c]	HO_2[d]	$h\nu$[e]
NO_2	2 days	12 hr	1 hr	2 hr	2 min
NO	4 days	1 min	3 min	20 min	-
HNO_3	180 days	-	-	-	-
SO_2	26 days	>200 yr	>4×10^4 yr	>600 yr	-
NH_3	140 days	-	-	-	-
Propane	19 days	>7,000 yr	-	-	-
n-Butane	9 days	>4,500 yr	9 yr	-	-
n-Octane	3 days	-	3 yr	-	-
Ethylene	3 days	9 days	3 yr	-	-
Propylene	11 hr	1.5 days	15 days	-	-
Acetylene	30 days	6 yr	>14 yr	-	-
Formaldehyde	3 days	>2×10^4 yr	210 days	23 days	4 hr
Acetaldehyde	1 day	>7 yr	50 days	-	60 hr
Benzaldehyde	2 days	-	60 days	-	-
Acrolein	1 day	60 days	-	-	-
Formic acid	50 days	-	-	-	-
Benzene	18 days	600 yr	>16 yr	-	-
Toluene	4 days	300 yr	9 yr	-	-
m-Xylene	11 hr	75 yr	2 yr	-	-
Phenol	10 hr	-	20 min	-	-
Naphthalene	1 day	>80 days	.[f]	-	-
2-Methylnaphthalene	5 hr	>40 days	.[f]	-	-
2,3-dimethylnaphthalene	4 hr	>40 days	.[f]	-	-
Acenaphthene	2 hr	>30 days	~3 hr	-	-
Acenaphthylene	2 hr	~50 min	13 min	-	-
Phenanthrene	9 hr	-	-	-	-
Anthracene	2 hr	-	-		-
Fluoranthene[g]	6 hr	-	.[f]	-	-
Pyrene[g]	6 hr	-	.[f]	-	-

[a] For 12-hr average concentration of OH radical of 1×10^6 molecule/cm^3.
[b] For 24-hr average O_3 concentration of 7×10^{11} molecule/cm^3.
[c] For 12-hr average NO_3 concentration of 2×10^8 molecule/cm^3.
[d] For 12-hr average HO_2 concentration of 10^8 molecule/cm^3.
[e] For solar zenith angle of 0 °.
[f] Lifetimes due to gas-phase reactions with a 12-hr average concentration of N_2O_5 of 2×10^{10} molecule/cm^3 are: naphthalene ~80 days, 2-methylnaphthalene ~35 days, 2,3-dimethylnaphthalene ~20 days, fluoranthene ~64 days, and pyrene ~20 days.
[g] Lifetimes calculated from kinetic data given in Atkinson et al., 1990.

Although the individual rate constants are known to a reasonable degree of accuracy (in general, to within a factor of two), the tropospheric concentrations of these key reactive species are much more uncertain. For example, the ambient concentrations of OH radicals at any given time and/or location are uncertain to a factor of at least 5, and more likely 10 (Atkinson,1988). The tropospheric diurnally and annually averaged OH radical concentrations are more certain, to possibly a factor of two. For this reason, the calculated lifetimes listed in Table 3-1 are approximate only and are valid for those reactive species concentrations which are listed in the footnotes. However, these data permit one to estimate the contribution of each of these atmospheric reaction to the overall rates of removal of most pollutants from the atmosphere.

As can be seen from Table 3-1, the major atmospheric loss process for most of the automotive emission constituents listed is by daytime reaction with OH radicals. For some pollutants, photolysis, reactions with ozone, and reactions with NO_3 radicals during nighttime hours are also important removal routes.

The atmospheric lifetimes do not take into consideration the potential chemical or biological importance of the products of these various reactions. For example, the reaction of gas-phase PAH with N_2O_5 appears to be of minor significance as a PAH loss process, but, as will be discussed later, more important as a route of formation of mutagenic nitro-PAH.

3.2.1.1 Reactions of NOx. Only the major atmospheric reactions of oxides of nitrogen are considered here; for detailed discussion of the chemistry of these important species, see Finlayson-Pitts and Pitts, 1986.

Oxides of nitrogen emitted by diesel engines include mainly NO with lesser amounts of NO_2. NO is easily oxidized to NO_2 in the reactions with HO_2 radicals and alkylperoxy radicals (the reaction of NO with O_2 is too slow at typical ambient concentrations of NO). In addition, NO reacts rapidly (Table 3-1) with ozone via reaction (1):

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad (1)$$

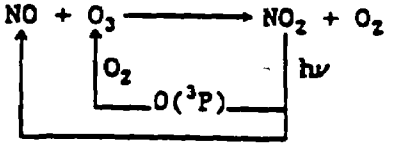
NO_2 is photolysed rapidly at wavelengths of <430 nm:

$$NO_2 + h\nu \rightarrow NO + O(^3P) \qquad (2)$$

The oxygen atom produced in this reaction reacts with O_2 forming ozone:

$$O_2 + O(^3P) \rightarrow O_3 \qquad (3)$$

The photolysis of NO_2 is the only known anthropogenic source of ozone, produced via reaction (2) and (3). With this series of reactions, NO, NO_2 and O_3 are in a photostationary state:



with

$$[O_3] = \frac{k_2\,[NO_2]}{k_1\,[NO]}$$

where k_1 and k_2 are the rate constants for the reactions (1) and (2), respectively, and brackets [ ] signify concentrations. This photostationary state is strongly affected by NO to NO_2 conversions caused by reactions involving organic compounds.

The important atmospheric reactions of NO_2 also include formation of NO_3 radicals and N_2O_5:

$$NO_2 + O_3 \rightarrow NO_3 + O_2$$

$$NO_2 + NO_3 \underset{}{\overset{M}{\rightleftharpoons}} N_2O_5$$

with N_2O_5 being in equilibrium with NO_2 and NO_3 radicals.

The other important atmospheric reactions of NO and NO_2 include nitrous and nitric acid formation, respectively, by reaction with OH radicals:

$$NO + OH \rightarrow HNO_2$$

$$NO_2 + OH \rightarrow HNO_3$$

3.2.1.2 Reactions of SO_2. Gas-phase reaction with OH radical is the dominant SO_2 atmospheric reaction process (Stockwell and Calvert, 1983):

$$SO_2 + OH \rightarrow HSO_3$$

followed by the formation of HO_2 radicals and H_2SO_4:

$$HSO_3 + O_2 \rightarrow HO_2 + SO_3$$
$$\downarrow H_2O$$
$$H_2SO_4$$

Since SO_2 is soluble in water, it undergoes scavenging by fog, cloud water, and raindrops. In aqueous system, SO_2 is readily oxidized to sulfate (Calvert and Stockwell, 1983).

3 2 1 3 Reactions of alkanes. Only a brief overview of the most important atmospheric reactions of alkanes is presented here; for detailed discussion consult Finlayson-Pitts and Pitts, 1986; Atkinson, 1988; Atkinson, 1990, and references therein.

Under atmospheric conditions, alkanes react with OH radicals during the daytime and with NO_3 radicals during nighttime hours:

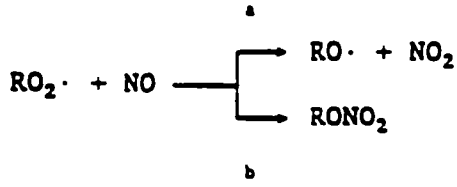
$$RH + OH \rightarrow R\cdot + H_2O$$
$$RH + NO_3 \rightarrow R\cdot + HNO_3$$

Alkyl radical, $R\cdot$, reacts with O_2 forming an alkylperoxy radical:

$$R\cdot + O_2 \overset{M}{\rightarrow} RO_2\cdot$$

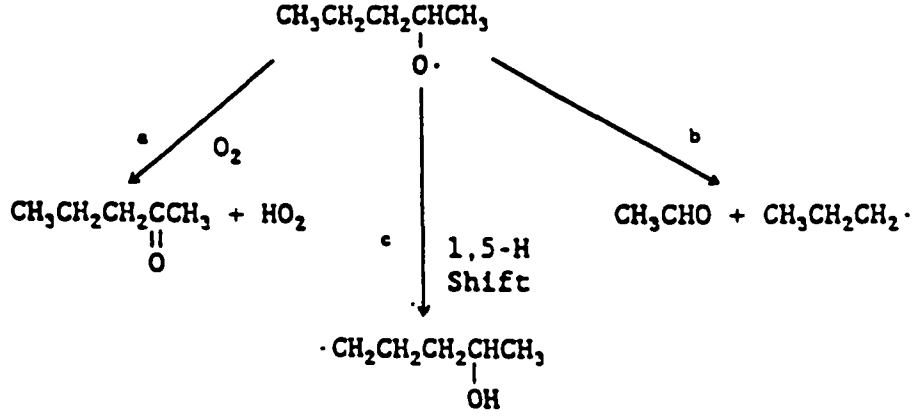
which, under polluted urban atmospheric conditions, reacts predominantly with NO by two pathways: (a) oxidation of NO to NO_2 and formation of alkoxy radical ($RO\cdot$)

-- this is the only significant path for the smaller ($\leq C_4$) radicals; and (b) the addition reaction to form stable alkyl nitrates -- this pathway becomes significant for larger alkyl peroxy radicals:



Alkoxy radical (RO·) reacts essentially by three routes: (a) with O_2, by abstraction of H atom from the neighboring carbon and formation of stable carbonyl compound and HO_2 radical, (b) unimolecular decomposition to form stable carbonyl compounds and free radicals, which will react further, probably by analogous routes as discussed for alkyl radicals above; (c) unimolecular isomerization by 1,4- or 1,5-hydrogen shift (if a hydrogen atom in appropriate position is available), forming α- or ß- substituted alkyl radical, which will react with O_2 as discussed above.

For example, for 2-pentoxy radical:



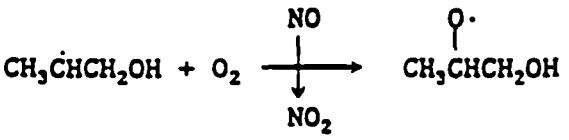
For the simplest alkoxy radicals, reaction with O_2 (pathway a) is predominant. For larger radicals, however, isomerization (c) and decomposition (b) may become significant, the relative importance of these two pathways depending on the structure of the radical. In all three cases free radicals are produced which then will carry on the chain reactions. The first-generation products include aldehydes, ketones, and alkyl nitrates, which can react further under atmospheric conditions.

3 2 1 4 Reactions of Alkene Lower molecular weight alkenes, such as ethylene, propylene, and isomeric butenes are present in exhaust from gasoline and (although in lower amount) diesel engines (see Table 2-2, Section 2.1.2). Gas-phase alkenes are removed from the troposphere by reaction with OH radicals, NO_3 radicals, and O_3 (Finlayson-Pitts and Pitts, 1986; Atkinson, 1988; Atkinson and Carter, 1984). Reactions with OH radicals are rapid (see Table 3-1) and proceed by OH radical addition to the double bond. For example, for propylene:

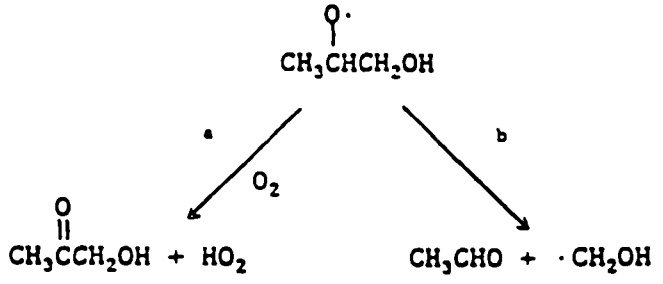
$$CH_3CH{=}CH_2 + OH \rightarrow \underset{\text{(a) } \sim 65\%}{CH_3CHCH_2OH} + \underset{\text{(b) } \sim 35\%}{CH_3\overset{\overset{OH}{|}}{C}HCH_2\cdot}$$

Formation of the more stable radical (a) is dominant.

Since the OH-olefin adduct is essentially an alkyl radical, it reacts further in a manner similar to alkyl radicals formed from the reaction of alkanes with OH radicals, e.g., by the addition of O_2 followed by reaction with NO. For example, for radical (a):



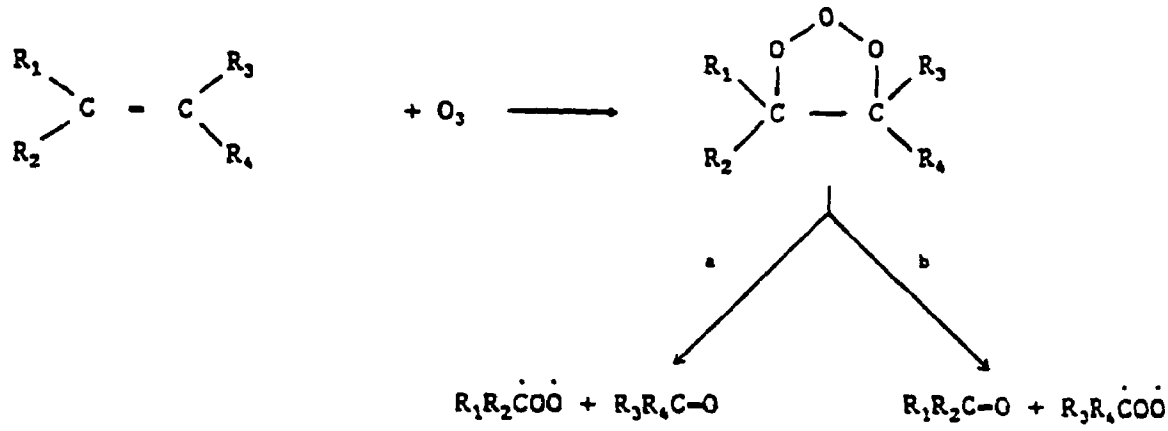
ß-hydroxyalkoxy radical is the major product. This radical, as discussed above for alkanes, can (a) react with O_2 or (b) decompose (isomerization is not important in the case of smaller alkenes):



The available data suggest that the decomposition (b) dominates over reaction with O_2. Thus, OH radical reactions with alkenes lead to the formation of aldehyde and/or ketones.

The NO_3 radical reaction becomes important under atmospheric conditions only for alkenes more reactive than propylene (see Table 3-1). Similarly to the OH radical reaction, this reaction proceeds through NO_3 radical addition to the double bond, followed by reaction with O_2 (see Finlayson-Pitts and Pitts, 1986; Atkinson 1988). Carbonyl compounds are formed as major products, but minor products (possibly dinitrates) are not well defined (Atkinson, 1988).

The reactions of alkenes with ozone compete with the daytime OH radical reaction (see Table 3-1). These reactions proceed by addition to the double bond, followed by rapid decomposition of so-called "ozonide" or "molozonide" into a carbonyl compound and an energy-rich biradical:

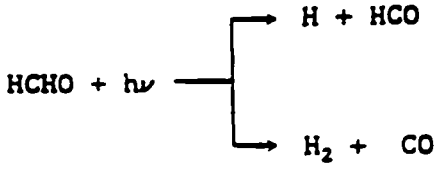


The importance of pathways (a) and (b) are assumed to be equal (Atkinson and Carter, 1984).

The major uncertainty concerns the atmospheric fate of the energy rich-intermediate, the so-called Criegee biradical. It is believed that some of the Criegee intermediates contain sufficient excess energy to spontaneously decompose. The remaining "thermalized" species are expected to react in the atmosphere with NO, NO_2, SO_2, H_2O vapor, CO, and carbonyl compounds, although the rate constants and mechanisms of these reactions are presently uncertain (Finlayson-Pitts and Pitts, 1986).

3.2.1.5 Reactions of oxygen containing organics. Diesel and gasoline vehicle exhausts contain aldehydes (mainly formaldehyde and acetaldehyde) and (most probably) carboxylic acids (See Section 2.1.2).

The major loss processes for aldehydes involve photolysis and reaction with OH radicals (see Table 3-1). Photolysis is an important loss process for formaldehyde, forming H atom and HCO radical in the first step:



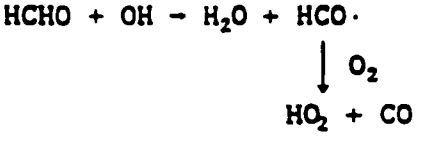
The rapid reaction of H atom and HCO radicals with O_2 produces HO_2 radicals:

$$H + O_2 \rightarrow HO_2$$

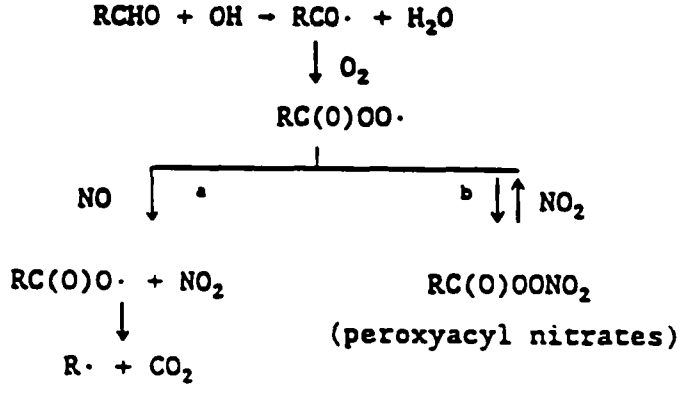
$$HCO + O_2 \rightarrow HO_2 + CO$$

The higher aldehydes also photodissociate, ultimately yielding HO_2 radicals.

The OH radical reaction with formaldehyde yields CO and HO_2:

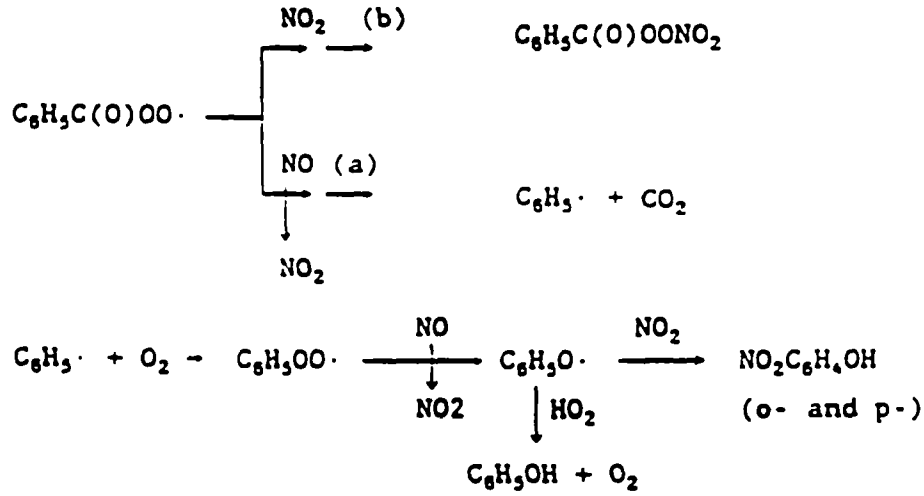


For higher aldehydes, the RCO radicals initially formed add O_2 to yield acylperoxy radical, which can react further with NO (pathway a) and NO_2 (pathway b):



Acetaldehyde forms peroxyacetyl nitrate (PAN) which has been shown to be a direct-acting mutagen toward Ames *Salmonella typhimurium* strain TA100 (Kleindienst et al., 1985) and is phytotoxic.

Benzaldehyde, the simplest aromatic aldehyde, forms peroxybenzoyl nitrate by the series of reactions analogous to pathway (b), above, and nitrophenols as a result of the reaction with NO, analogous to pathway (a), above:



Carboxylic acids react with OH radicals under atmospheric conditions (see Table 3-1). For formic acid, hydrogen atoms are produced (Atkinson 1988):

$$HC(O)OH + OH \rightarrow H_2O + CO_2 + H$$

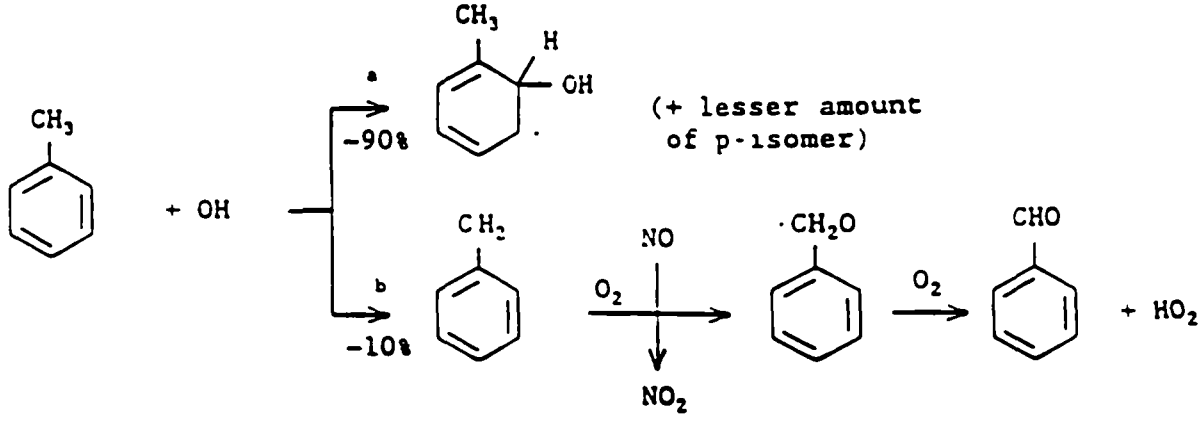
For the higher carboxylic acids, reaction products are presently unknown.

3.2.1.6 Reactions of monocyclic aromatic compounds. The monocyclic aromatic compounds are removed from the atmosphere solely by reactions with OH radicals (see Table 3-1). These reactions proceed by two pathways (Finlayson-Pitts and Pitts, 1986; Atkinson, 1988):

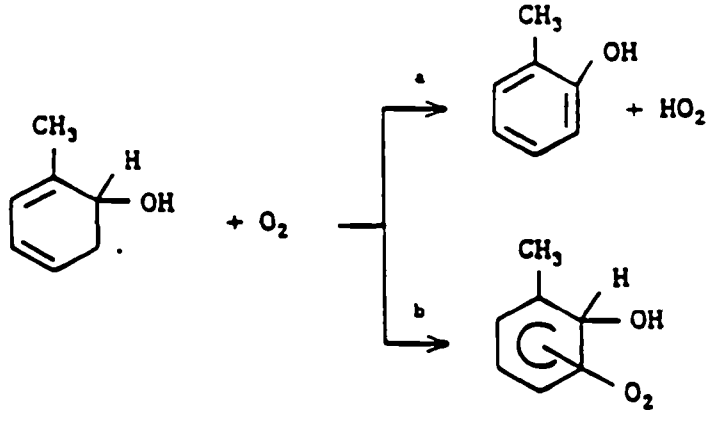
(a) a major pathway by OH radical addition to the aromatic ring;

(b) a minor pathway by H atom abstraction, either from the aromatic ring (in the case of benzene) or from an alkyl group (in the case of alkyl-substituted aromatics). This latter pathway leads to the formation of aromatic aldehydes and is analogous to OH radical reaction of alkanes.

For example, for toluene:



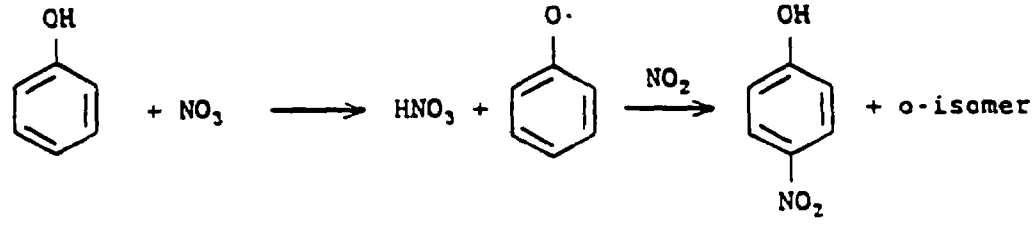
The products arising from the OH radical addition pathway (a) are not well known. Reaction with O_2, again occurring by two pathways, is expected to predominate:



Pathway (a) yields phenolic compounds and, for toluene, accounts for ~20% of the overall reaction yield (Atkinson, 1988). The major reactions involve ring

cleavage (opening), leading to a variety of bifunctional products (Finlayson-Pitts and Pitts, 1986).

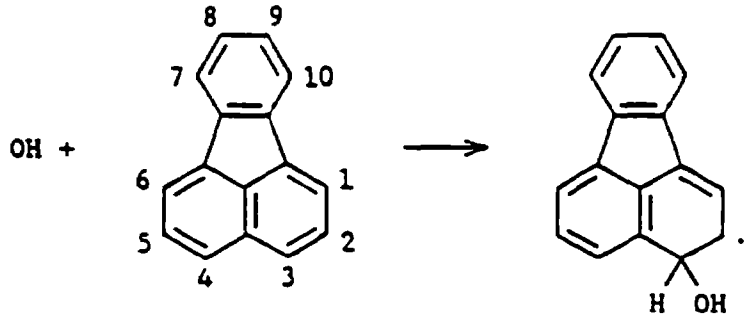
For phenolic compounds, in addition to OH radical reaction (proceeding mainly by initial OH radical addition to the ring), the NO_3 radical reaction yielding nitrophenols, appear to be important (Atkinson, 1988):



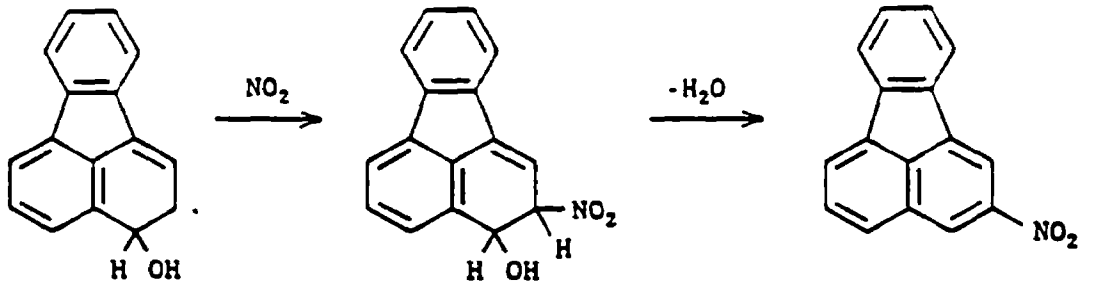
3 2 1 7 Reactions of polycyclic aromatic compounds (PAH). As was discussed in Section 2.4, two- to four-ring PAH emitted from diesel and spark-ignition engines are distributed between gas and particle phases. For those PAH present in the gas phase, the reaction with OH radical is predominant, leading to atmospheric lifetimes of a few hours or less (see Table 3-1). The nighttime gas-phase reaction with N_2O_5 is of minor significance as a PAH loss process, but (as will be discussed below) may be important as a formation route of mutagenic nitro-PAH. In addition, for the PAH containing cyclopenta-fused ring, such as acenaphthene, acenaphthylene, and acephenanthrylene, the NO_3 radical reaction can be an important gas-phase loss process during nighttime hours.

Relatively few product data are available concerning these gas-phase reactions. It has recently been shown that, in the presence of NO_x, the OH radical reactions with naphthalene, 1- and 2-methylnapnthalene, acenaphthylene, biphenyl, fluoranthene, pyrene, and acephenanthrylene lead to the formation of nitroarenes (Arey at al., 1986; 1989; Sweetman et al., 1986; Atkinson et al., 1987; Zielinska et al., 1986; 1988; 1989a). The postulated reaction pathway

involves initial OH radical addition to the most reactive ring position; for example, C-3 position for fluoranthene (Pitts et al., 1985a):



followed by NO_2 addition in the C-2 position. Subsequent elimination of water results in 2-nitrofluoranthene formation:



The analogous reaction sequence for pyrene produces 2-nitropyrene (Pitts et al., 1985a). In contrast, the electrophilic nitration reactions of fluoranthene, and pyrene, involving NO_2^+ ion, produces mainly 3-nitrofluoranthene from fluoranthene and 1-nitropyrene from pyrene.

The gas-phase reactions of N_2O_5 with naphthalene, 1- and 2-methylnaphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene, and pyrene yield, in general, the same nitro-PAH isomers as OH radical reaction, but with different yields. For example, the same 2-nitrofluoranthene is produced from both OH radical and N_2O_5 gas-phase fluoranthene reactions, but the reaction with N_2O_5 produces a much higher yield. The postulated reaction pathway involves initial NO_3 radical addition to the most reactive position, followed by NO_2

addition to the neighboring position and elimination of HNO_3 (Zielinska et al., 1986):

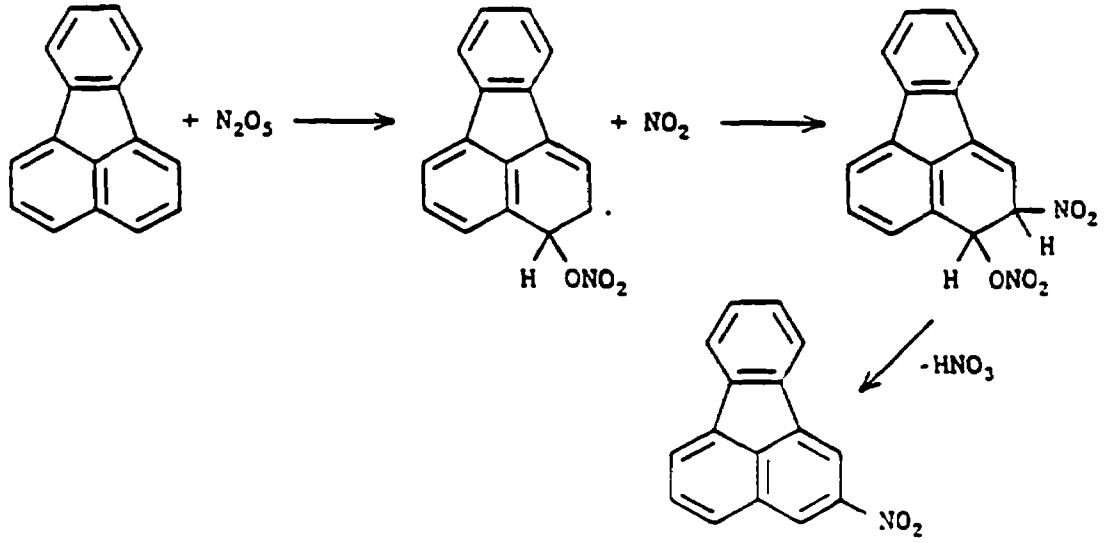


Table 3-2 summarizes the nitroarene product data for OH radical initiated and N_2O_5 gas-phase reactions with several PAH studied to date in environmental chambers (Arey et al., 1989; Zielinska et al., 1990). As will be discussed in Section 4.3 of this Chapter, generally the same nitro-PAH isomers as those formed from OH radical and N_2O_5 reactions are observed in ambient air samples.

3.2.2 Particulate-Phase Reactions

As discussed in Section 2.2.3 of this Chapter, thousands of organic compounds present in diesel exhausts are partitioned essentially totally into the particulate phase under atmospheric conditions. The following chemical processes are likely to contribute to the degradation of these compounds in the troposphere (Atkinson, 1988):

- photolysis
- reaction with O_3
- reaction with N_2O_5 during nighttime hours
- reaction with NO_2 during nighttime and daytime hours

Table 3-2

Summary of the Nitroarenes Produced from the Gas-Phase OH Radical-Initiated and N_2O_5 Reactions and Electrophilic Nitration of PAH

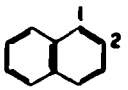
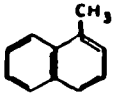
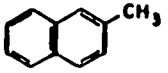
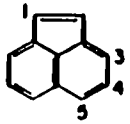
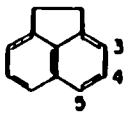
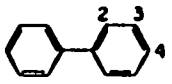
PAH	Structure	Position of Nitration (Yield) in Reaction with OH	Position of Nitration (Yield) in Reaction with N_2O_5	Position of Electrophilic Nitration
Naphthalene		1-(0.3%); 2-(0.3%)	1-(17%), 2-(7%)	1->>2-
1-Methylnaphthalene		5->4-≥6-≥3-≥7--2->>8- Total yield (~0.4%)	3->5-≥4-≥8--6->7-≥2- Total yield (~30%)	4->2->5->8->7--3->6-
2-Methylnaphthalene		5->6--7--4--8->>3->1- Total yield (~0.2%)	4->1--5-≥8--3--7--6- Total yield (~30%)	1->>8->4->6->5->3->7-
Acenaphthylene		4- (2%)	None observed	1-
Acenaphthene		5->3->4- Total yield (~0.2%)	4-(40%), 3-(~2%), 5-(~2%)[a]	3-; 5-
Biphenyl		3- (5%)	No reaction observed	2-; 4-

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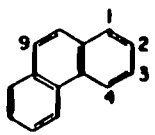
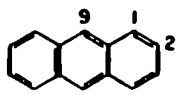
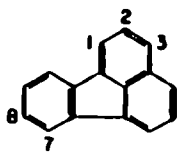
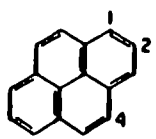
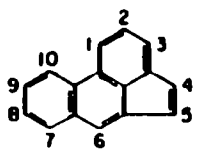
Table 3-2 (continued)

Summary of the Nitroarenes Produced from the Gas-Phase OH Radical-Initiated and N_2O_5 Reactions and Electrophilic Nitration of PAH

PAH	Structure	Position of Nitration (Yield) in Reaction with OH	Position of Nitration (Yield) in Reaction with N_2O_5	Position of Electrophilic Nitration
Phenanthrene		Two isomers (not 9-nitrophenanthrene) Total yield (≤0 1%)	Four isomers (including 9-nitrophenanthrene) Total yield (<1%)	9->3-; 2-; 1-
Anthracene		1-, 2- Total yield (~0 2%)	1-, 2- Total yield (<2%)	9-
Fluoranthene		2-(3%); 7-(~0.15%); 8-(~0.15%)	2-(~25-30%)	3->8->7->1-
Pyrene		2-(~0 25%), 4-(~0 045%)	4-, 2- Total yield (<1%)	1-
Acephenanthrylene		Two isomers (not 4- or 5-nitro-) Total yield (~0 1%)	None observed	4-, 5-

[a] Concurrent NO_3 radical reaction will dominate over N_2O_5 reaction in ambient air

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- reaction with H_2O_2
- reaction with HNO_3, HNO_2 and H_2SO_4

However, the atmospheric lifetimes of particle-bound organic compounds are not well known. This is partially due to the fact that these chemical processes are dependent upon the nature of the substrate (see, for example, Behymer and Hites, 1985; 1988) and that many of the laboratory studies have been done using atmospherically unrealistic adsorbents, such as glass fiber and Teflon-coated glass-fiber filters, silica gel, and alumina. The extrapolation of sometimes contradictory results reported by different laboratories to atmospherically realistic conditions presents major problems.

The atmospheric fate of particle-bound PAH has received much attention since their potential toxicity was first realized. In their recent publication, Behymer and Hites (1988) define two opposite schools of thought on this subject. One school, which they call the "Fast School," says that particle-bound PAH degrade quickly in the atmosphere with lifetimes as short as few hours (see, for example Kamens et al., 1988; Nielsen 1988; and references cited in Behymer and Hites, 1988). The other school, called the "Slow School" (which the authors represent), says that PAH degrade slowly, if at all, in the atmosphere and eventually deposit on soil or water. The "Slow School" conclusion is supported by the studies of marine and lacustrine sediments (the ultimate environmental sinks of PAH) which have shown that the relative abundances of PAH, even at the most remote locations, are similar to those in combustion sources and in air particulate matter (Laflamme and Hites, 1978; Hites et al., 1980; McVetty and Hites, 1988).

3.2.2.1 Photooxidation of particulate PAH. Laboratory studies of photolysis of PAH adsorbed on 18 different fly ashes, carbon black, silica gel, and alumina (Behymer and Hites, 1985; 1988) and several coal stack ashes (Yokley et al., 1986; Dunstan et al., 1989) showed that the extent of PAH photodegradation depended very much on the nature of the substrate to which they are adsorbed. The dominant factor in the stabilization of PAH adsorbed on fly ash was the color of the fly ash, which is related to the amount of black carbon present. It appeared that PAH were stabilized if the carbon content of the fly ash was greater than ~5%. On black substrates, half-lives of PAH studied were on the order of several days (Behymer and Hites, 1988).

Similar conclusions were reached from studies of photolysis of PAH adsorbed on coal stack ashes (Yokley et al., 1986; Dunstan et al., 1989). The relative quantity of carbon in coal ash was the main factor determining the extent of photochemical degradation of pyrene and benzo(a)pyrene adsorbed on the surface. In addition, in coal ashes that contained a relatively large quantity of iron, the magnetic particles played a minor role in stabilizing adsorbed pyrene toward photodegradation (Dunstan et al., 1989).

On the other hand, the environmental chamber studies of Kamens and co-workers (1988) on the daytime decay of PAH present on residential wood smoke particles and on gasoline internal combustion emission particles showed PAH half-lives of the order of 1 hr at moderate humidities and temperatures. At very low-angle sunlight, very low water-vapor concentration, or very low temperatures, PAH daytime half-lives increased to a period of days.

In addition, the atmospheric studies of Nielsen (1988), carried out in rural areas during the winter and early spring when ambient temperatures and concentrations of NO_2 and O_3 were low, showed evidence for atmospheric decay of

more reactive PAH, such as benzo(a)pyrene and cyclopenteno(cd)pyrene. Although no estimation of these PAH lifetimes was given, the author concluded that the decay appeared to be relatively fast.

Due to the limited understanding of the mechanisms of these complex heterogenous reactions, it is presently impossible to draw any firm conclusion concerning the photostability of particle-bound PAH in the atmosphere. Since diesel particulate matter contains a relatively high quantity of elemental carbon (see Section 2.2.1), it is reasonable to assume that PAH adsorbed onto these particles should be relatively stable under standard atmospheric conditions. Clearly, additional comprehensive and systematic investigation of adsorbed-phase reactions of PAH is needed.

3.2.2.2 Nitration of particulate PAH under simulated atmospheres. Since 1978 when Pitts and co-workers (Pitts et al., 1978) first demonstrated that benzo(a)pyrene (BaP) deposited on glass-fiber filters and exposed to air containing 0.25 ppm of NO_2 with traces of HNO_3 formed nitro-BaP, numerous studies of the heterogenous nitration reactions of PAH adsorbed on a variety of substrates in different simulated atmospheres have been carried out (see, for example, Finlayson-Pitts and Pitts, 1986). It has been shown that the rate of PAH nitration in a NO_2/HNO_3 system depends on the substrate to which the PAH are adsorbed (Jäger and Hanus, 1980) and on the reactivity of particular PAH (Nielsen, 1984).

It has also been shown that certain PAH deposited on glass-fiber and Teflon-impregnated glass-fiber filters react with gaseous N_2O_5 yielding their nitro derivatives (Pitts et al., 1985b, 1985c). The nitro-PAH isomers formed from the parent PAH are the same as those formed from electrophilic nitration

reactions involving NO_2^+ ions. Thus, the most abundant isomers formed were 1-nitropyrene from pyrene, 6-nitro-B(a)P from BaP, 3-nitroperylene from perylene. For fluoranthene, 3-, 8-, 7- and 1-nitrofluoranthene isomers were formed in approximately equal amounts in N_2O_5 reactions, whereas the nitrofluoranthene isomer distribution from electrophilic nitration reaction is 3-> 8->> 7-≥ 1-nitrofluoranthene. However, no 2-nitrofluoranthene (the sole isomer formed from gas-phase N_2O_5 reaction) was produced from this adsorbed-phase reaction (Pitts et al., 1985c). It was speculated that N_2O_5 becomes ionized on the filter surface prior to the reaction with fluoranthene, but the resulting NO_2^+ ion is not "free" nitronium ion, i.e., not completely dissociated (Zielinska et al., 1986).

Based on these laboratory studies, it has been proposed that some nitro-PAH detected in ambient particles may be formed from the reaction of the parent PAH with gaseous co-pollutants in the atmosphere, or during the collection of particulate matter, or both (Pitts et al., 1978, 1985a; Jäger and Hanus, 1980; Brorstrom et al., 1983). However, the extrapolation of the data obtained under laboratory conditions to the ambient atmosphere requires several major assumptions. These include, for example, the assumptions that substrate effect, PAH concentration, the presence of co-pollutants, relative humidity, etc., have no major impact on PAH nitration reactivities. If so, the available data indicate that the nitration of particle-bound PAH with NO_2/HNO_3 and N_2O_5 is probably not significant under atmospheric conditions (Pitts et al., 1985c). However, this may not always be the case in airsheds with high NO_2 and nighttime N_2O_5 concentrations.

The formation of nitro-PAH during sampling may be an important problem for diesel particulate matter collection, since NO_2 and HNO_3 are present in relatively high concentrations. However, Schuetzle (1983) concluded that the

artifactual formation of 1-nitropyrene during dilution tube sampling accounts for less than 10-20% of the total 1-nitropyrene present in diesel particles, if the sampling time is less than 23 min (one FTP cycle) and if the sampling temperature is not higher than 43 °C.

The formation of nitroarenes during ambient hi-vol sampling conditions has been reported to be minimal, at least for the most abundant nitropyrene and nitrofluoranthene isomers (Arey et al., 1988a).

3 2 2 3 Ozonolysis of particulate PAH. Numerous laboratory studies have shown that PAH deposited on combustion-generated fine particles and on model substrates undergo reaction with ozone (see, for example, Katz et al., 1979; Pitts et al., 1980, 1986; Van Vaeck and Van Cauwenberghe, 1984, Finlayson-Pitts and Pitts, 1986, and references therein). The kinetics of the dark reaction of several PAH deposited on model substrates toward O_3 has been shown to be relatively fast under simulated atmospheric conditions (Katz et al., 1979; Pitts et al., 1980, 1986) Half-lives of the order of one to several hours were reported for the more reactive PAH, such as BaP, anthracene, and benz(a)anthracene (Katz et al., 1979)

The reaction of PAH deposited on diesel particles with 1.5 ppm of O_3 under hi-vol sampling conditions was shown to be relatively fast, and the approximate half-lives of the order of 0.5 to 1 h were reported for most PAH studied (Van Vaeck and Van Cauwenberghe, 1984). The most reactive PAH included BaP, perylene, benz(a)anthracene, cyclopenta(cd)pyrene, and benzo(ghi)perylene. The benzofluoranthene isomers are the least reactive of the PAH studied and benzo(e)pyrene is less reactive than its isomer BaP. The implications of this study for the hi-vol sampling of ambient POM are important: reaction of PAH with

O_3 could possibly occur under hi-vol sampling conditions during severe photochemical smog episodes, when the ambient level of O_3 is high. However, the magnitude of this artifact is difficult to assess based on available data.

Exposures of PAH adsorbed on filters and ambient particulate matter to ambient levels of O_3 in an environmental chamber under "passive" conditions, more nearly resembling atmospheric transport (as opposed to filtration type experiments analogous to hi-vol sampling of particles), were also carried out (Pitts et al., 1986). These experiments showed that significant degradation of more reactive PAH adsorbed on ambient particulate matter, such as BaP, pyrene, and benz(a)anthracene, may occur in ozone-polluted atmospheres.

3.3 Physical Removal Processes

3.3.1 Dry Deposition

Dry deposition is the removal of particles and gases from the atmosphere through the delivery of mass to the surface by nonprecipitation atmospheric processes, and the subsequent physical attachment to, or chemical reaction with, the surfaces, (e.g., vegetation, soil, water, or the built environment) [Dolske and Gatz, 1985]. It should be noted that the surface itself may be wet or dry; the term "dry deposition" refers to the mechanism or transport to the surface, not to the nature of the surface itself. Dry deposition plays an important role as a removal mechanism of pollutant in the absence of precipitation. Even in remote locations such as Siskiwit Lake, located on a wilderness island in northern Lake Superior, the dry deposition of aerosol was found to exceed the wet removal mechanism by an average ratio of 9:1 (McVeety and Hites, 1988).

Dry deposition is usually characterized by a deposition velocity, V_d, which is defined as the flux (F), or deposition rate, of the species S to the surface divided by the concentration [S] at some reference height (generally 1 m):

$$V_d = \frac{F}{[S]}$$

The amount of species deposited per unit area per second in a given geographical location, that is, the flux, can be either calculated, if the deposition velocity and the pollutant concentration are known, or measured experimentally. The deposition velocity depends on the specific gaseous or particle species, the properties of the surface to which the species is being deposited, and the reference height. It also depends on a micrometeorological process that transports the species to the surface (see, for example, Finlayson-Pitts and Pitts, 1986, and references therein, for more detailed discussion).

For particles, the deposition velocities depend on the particle size, exhibiting a minimum for particles of mean diameter of ~0.1 to ~1 μm. Table 3-3 gives the average calculated lifetimes of atmospheric particles as a function of their diameter (from Atkinson, 1988):

Table 3-3

Average Atmospheric Lifetimes of Particles Due to Dry Deposition[a]

Diameter (μm)	Lifetime (days)
0.002	0.01
0.02	1
0.2	10
2	10
20	1
200	0.01

[a]From Atkinson (1988); see this for original references.

Table 3-4 gives some selected examples of dry deposition velocity for several inorganic and organic species (from Dolske and Gatz, 1985, with grass as the surface, except as noted).

Table 3-4

Examples of Dry Deposition Velocities[a]

Depositing Species	Mean Deposition Velocity (cm/sec)
Ozone	0.49
Particulate sulfur	0.17
Particles:	
0.18 μm median diameter	0.16
0.25 μm median diameter	0.35
SO_2	2.1
HNO_3	2.5
Benzeno(ghi)perylene[b]	0.99
Indeno[1,2,3-cd] pyrene[b]	0.99

[a]From Dolske and Gatz, 1985, with grass as the surface, except as noted.
[b]From McVeety and Hites, 1988, with water as the surface.

However, due to the differences in meteorology, nature of surfaces, diurnal variation, and measurement uncertainties, the reported values for deposition velocities of a given species can differ by more than an order of magnitude. It is important to note, that for certain chemicals that have relatively slow gas-phase chemical removal rates, such as SO_2 and HNO_3, dry deposition can be the major loss process under typical atmospheric conditions.

3.2.2 Wet Deposition

Wet deposition encompasses all processes by which airborne pollutants are transported to the Earth's surface in aqueous form (i.e., in rain, snow, or fog).

The mechanisms of wet removal from the atmosphere may be very different for particle-associated compounds and for gas-phase compounds. However, since many organic compounds are partitioned between the aerosol and vapor phase (as discussed in Section 2.4), both processes of gas and particle scavenging may be important for a given compound (Ligocki et al., 1985a, 1985b; Bidleman, 1988). When there is no exchange of material between the particulate and dissolved phases in the rain, the total scavenging of a given compound can be expressed as (Pankow et al., 1984):

$$W = W_g (1-\phi) + W_p\phi$$

where W is the overall scavenging ratio:

$$W = \frac{[\text{rain, total}]}{[\text{air, total}]}$$

W_g is the gas scavenging ratio:

$$W_g = \frac{[\text{rain, dissolved}]}{[\text{air, gas}]}$$

W_p is the particle scavenging ratio:

$$W_p = \frac{[\text{rain, particulate}]}{[\text{air, particulate}]}$$

and ϕ is the fraction of the atmospheric concentration which is associated with particles.

Particle scavenging is a complex process which depends upon the meteorological conditions in the cloud as well as the size and chemical composition of the aerosol particles. The simplest model for in-cloud particle scavenging involves nucleation scavenging followed by coalescence of the cloud droplet into raindrops. As many as $\sim 10^6$ cloud droplets of ~10 μm diameter must combine to form one 1 mm raindrop. Hence, scavenging ratios under these conditions are expected to be of the order 10^6. However, this process alone seldom produces precipitation.

In cold clouds, ice crystals grow by vapor accretion and by collection of supercooled droplets (riming) Scavenging ratios may be considerably lower than 10^6 under these conditions. In the case of below-cloud scavenging, W_p values have been estimated to be 10^3 - 10^5 for 0.01 -1.0 μm particles (Slinn et al., 1978). From these data one may expect to observe overall particle scavenging ratios in the range of 10^3 - 10^6.

Ligocki and co-workers (Ligocki et al., 1985a, 1985b) measured gas- and particle-scavenging ratios for a number of organic compounds, including PAH and their derivatives. Table 3-5 gives mean gas, particle and overall scavenging ratios for measured neutral organic compounds. It can be seen from this table that particle scavenging ratios range from 10^2 to 10^5, whereas gas scavenging ratios range from 22 to 10^5. Gas scavenging dominates over particle scavenging for compounds of lower molecular weights (mw < 252 for PAH). Particle scavenging dominates for the alkanes, which are essentially insoluble in water.

The complexity of liquid-phase inorganic acid formations from gaseous precursors and the problems of acid rain and acid fog are beyond the scope of this chapter and are not discussed here (see, for example Finlayson-Pitts and Pitts, 1986, and references therein for more information).

Table 3-5

Mean Particle, Gas, and Overall Scavenging Ratios for Neutral Organic Compounds[a]

Compound	Mean ϕ[b]	Mean W_p	Mean W_g	Mean W[c]	Dominant scav mech.[d]
Toluene	0.0	0.0	22	22±5	g
1,2,4-Trimethylbenzene	0.0	0.0	27±9	27±9	g
Ethylbenzene	0.0	0.0	27±11	27±11	g
mtp-Xylene	0.0	0.0	33±17	33±17	g
o-Xylene	0.0	0.0	35±15	35±15	g
Naphthalene	0.0	0.0	250±73	250±73	g
2-Methylnaphthalene	0.0	0.0	250±78	250±78	g
1-Methylnaphthalene	0.0	0.0	330±100	330±100	g
Diethylphthalate	0.0	NA	20,000	20,000	g
Dibenzofuran	0.008	11,000	930	1000	g
Fluorene	0.009	15,000	1500	1600	g
Phenanthrene + Anthracene	0.011	17,000	3300	3500	g
0-Fluorenone	0.021	15,000	11,000	11,000	g
Methylphenanthrenes	0.027	13,000	2500	2800	g
Fluoranthene	0.053	11,000	6300	6600	g
Pyrene	0.071	9300	5900	6100	g
Eicosane	0.14	40,000	NA	5600	p
9,10-Anthracenedione	0.21	2400	27,000	22,000	g
Dioctylphthalate	0.56	36,000	20,000	30,000	p
Docosane	0.61	27,000	NA	17,000	p
Chrysene	0.71	2600	18,000	7000	g
Benz[a]anthracene	0.75	1300	12,000	4000	g
Benzo[e]pyrene	0.97	2000	5800	2100	p
Benzo[a]pyrene	0.98	1700	NA	1700	p
Benzo[b+j+k]fluoranthene	0.98	2200	7400	2300	p
Perylene	1.0	1800	NA	1800	p
Tricosane	1.0	22,000	NA	22,000	p
Teracosane	1.0	16,000	NA	16,000	p
Benzo[ghi]perylene	1.0	3100	NA	3100	p
Coronene	1.0	5900	NA	5900	p

[a]From Ligocki et al. (1985a, 1985b).
[b]ϕ = [aerosol]/[vapor + aerosol].
[c]$W = W_p\phi + W_g(1-\phi)$.
[d]g = gas.
p = particle.
NA = not available.

4.0 ATMOSPHERIC CONCENTRATIONS OF PRIMARY DIESEL EMISSIONS AND THEIR TRANSFORMATION PRODUCTS

Most of the data collected on vehicle emissions are from laboratory studies using dynamometer/dilution tube measurements. The relevance of these measurements to the atmosphere is always a question, since emissions from vehicles on the road have much higher dilution ratios ($\sim 10^3$ versus 10), are collected at lower temperature, are composed of a large number of individual vehicle exhausts, have longer residence times (seconds to days, versus ~5 seconds) and, as discussed in Section 3, interact with ambient air pollutants.

Pierson and co-workers (Pierson et al., 1983; Salmeen et al., 1985) conducted field experiments in the Allegheny Mountain Tunnel of the Pennsylvania Turnpike, to address this problem. They found that the diesel-produced particulate matter at tunnels was, in general, very similar to that encountered in dilution-tube studies with respect to total particulate matter emission rates, percentage extractables, hydrocarbon molecular weight distribution, HPLC profiles, particle size distribution, elemental compositions and extract mutagenicities. However, these findings did not preclude the possibility of substantial differences in detailed chemical compositions. Indeed, the concentration of 1-nitropyrene in the extract of particulate samples collected in the Allegheny Mountain Tunnel was reported to be lower than would be predicted on the basis of laboratory dilution tube measurements either for diesel or spark-ignition vehicles (Gorse et al., 1983).

The data on organic compound concentrations in airsheds heavily impacted by motor vehicle emissions (tunnels, roadsides, etc.) are reviewed below. The possibility of using these data to distinguish emissions from different sources is discussed in Section 4.4.

4.1 Volatile Organic Compounds (VOC) Attributable to Traffic

Individual volatile hydrocarbons and aldehydes were measured along a section of U.S. Highway 70 near Raleigh, NC (Zweidinger et al., 1988). Traffic volume during sampling was determined by visual counting (~ 1050 ± 10% vehicles/h in each direction) and was classified into four groups: light-duty, including gasoline and diesel vehicles through class 2 trucks; heavy-duty gasoline, heavy-duty diesel; and motorcycles. Typical distributions were 91.5%, 3.2%, 5.1% and 0.2%, respectively.

Table 4-1 lists the mean concentrations from four roadsides for selected hydrocarbons and aldehydes, expressed in ppbC and as a percent contribution of individual hydrocarbons and aldehydes to total nonmethane hydrocarbons (TNMHC) and total aldehydes, respectively.

The roadside VOC distribution was compared to dynamometer/dilution tube results on in-use vehicles, which were weighted in an attempt to reflect the same model year distribution as observed on the roadway (Sigsby et al., 1987, see also Table 2-2, Section 2.1.2). The two sets of data were similar in that the different driving cycles, like the different sampling sites, generally show no significant differences in the distribution of hydrocarbons or aldehydes on a percent of total basis. There were, however, differences observed between the sets of data, particularly for the contribution of combustion products (i.e., hydrocarbons below C_4 and aldehydes). For the roadside study, ethylene, formaldehyde, and acetaldehyde were lower, while acetylene was higher than in the dynamometer study. However, noncatalyst vehicles, which constituted 15% of all light-duty vehicles in the roadside study, as well as light- and heavy-duty diesel vehicles and trucks, were not included in the dynamometer study.

Table 4-1

Concentrations of Individual Hydrocarbons and Aldehydes Measured in the Raleigh (NC) Roadside Study[a]

Compound	Concentration [ppbC]	Percent Contribution[c,d]
Hydrocarbons		
Ethane	16.3	1.81
Ethylene	64.3	7.15
Acetylene	50.9	5.65
Propane	7.9	0.88
Propylene	22.6	2.51
n-Butane	15.8	1.75
1-Butene	5.7	0.64
n-Pentane	25.4	2.82
iso-Pentane	53	5.89
Methylcyclopentane	10.4	1.15
Methylcyclohexane	4.7	0.53
n-Decane	3.0	0.33
Benzene	29	3.23
Toluene	59.3	6.60
m- and p-Xylenes	53.1	5.90
o-Xylene	12.7	1.41
Ethylbenzene	12.0	1.33
TNMHC[b]	900	100
Total paraffins	369.2	41.0
Total olefins	164.5	18.2
Total aromatics	252.0	28.0
Total unidentified NMHC	63.6	7.1
Aldehydes		
Formaldehyde	6.7	41.05
Acetaldehyde	3.0	18.4
Acrolein	1.2	7.3
Benzaldehyde	2.3	13.88
Total aldehydes	16.38	100

[a] From Zweidinger et al., 1988.
[b] TNMHC: total nonmethane hydrocarbons.
[c] Percents based on ppbC.
[d] Percent contribution of individual hydrocarbons to TNMHC and of individual aldehydes to total aldehydes.

4.2 Polycyclic Aromatic Hydrocarbons

Particulate and vapor phase samples were collected from the traffic passing through the Baltimore Harbor Tunnel and analyzed for PAH and related compounds (Benner et al., 1989). High-volume air samplers equipped with Teflon filters backed by polyurethane foam (PUF) plugs were used for sample collection. There was no breakdown of traffic into numbers of diesel- and gasoline-fueled vehicles.

The range of particle-phase PAH concentrations and the mean particle- and vapor-phase PAH concentrations for 48 samples collected in the Baltimore Tunnel are tabulated in Table 4-2. The ratios of mean particle-phase PAH concentrations to that of benzo(e)pyrene (BeP), which is considered to be a nonreactive PAH, are also given in this table.

As can be seen from this table, alkyl-substituted phenanthrenes in the Tunnel samples had relatively high concentrations compared with those of the parent compound. This suggests a significant contribution from diesel vehicle emissions (particularly diesel-fueled trucks), since extracts of diesel particulate matter are known to have significant concentrations of methyl and dimethylphenanthrenes (see Table 2-5, Section 2.2.3, and also Yu and Hites, 1981).

Factor analysis was applied to the Tunnel data in an attempt to identify factors associated with different types of vehicles, with two factors being obtained. The alkylated phenanthrenes loaded significantly on factor 1, suggesting the diesel vehicles as the source of these compounds. Several of the higher molecular weight PAH loaded onto factor 2, which may be associated with the contribution of gasoline-fueled emissions in the Tunnel.

Table 4-2

Particle- and Vapor-Phase PAH Concentrations for Baltimore Harbor Tunnel Samples[a]

Compound	Concentration, ng/m³			
	Range, particles	Mean, particles	Mean, vapor[b]	Ratio[d] to BeP
Phenanthrene	4.3 - 56	18	132	4.3
Anthracene	0.6 - 12	2.9	18	0.6
3-Methyl-phenanthrene	3.9 - 58	13.9	70	3.3
2-Methyl-phenanthrene	5.3 - 74	19	-	4.6
2-Methylanthracene	0.6 - 12	3.0	5.3	0.7
9-and 4-Methyl phenanthrene and 4H-cyclopenta(def)-phenanthrene	4.7 - 50	12.9	71	3.0
1-Methylphenanthrene	2.6 - 43	9.8	43	2.3
2,6-Dimethyl-phenanthrene	4.7 - 62	14	30	3.4
2,7-Dimethyl-phenanthrene	3.4 - 38	9.2	16	2.2
1,3-, 2,10-, 3,9- and 3,10-Dimethyl-phenanthrene	9.5 - 119	26	61	6.3
1,6- and 2,9-Di-methylphenanthrene	4.5 - 63	14	27	3.3
1,7-Dimethyl-phenanthrene	3.9 - 41	10.2	20	2.4
2,3-Dimethyl-phenanthrene	3.5 - 41	9.3	16	2.2
Fluoranthene	6.4 - 69	20	16	4.5
Pyrene	9.7 - 76	27	26	6.3
Benzo(ghi)-fluoranthene	3.2 - 26	9.6	ND[c]	2.1
Cyclopenta(cd)-pyrene	7.6 - 65	20	ND	4.6
Benz(a)anthracene	1.9 - 29	7.6	ND	1.5
Chrysene/Tri-phenylene	2.9 - 47	12	ND	2.4
Benzofluoran-thenes [b,j,+k]	2.2 - 44	10.6	ND	2.1
Benzo(e)pyrene	1.5 - 19	5.0	ND	1
Benzo(a)pyrene	1.3 - 26	5.8	ND	1.1
Indeno[1,2,3-cd]-pyrene	0.3 - 15	4.6	ND	0.9
Benzo(ghi)perylene	1.8 - 18	8.0	ND	1.6
Coronene	1.0 - 10	4.7	ND	0.9

[a] From Benner et al., 1989.
[b] Mean concentrations of PAH collected on PUF plug (calculated from data given in Table III of Benner et al., 1989).
[c] None detected.
[d] Mean ratios.

Ambient air sampling for PAH was also conducted during a summertime photochemical air pollution episode in Glendora, CA, at a site situated less than 1 km from the heavily traveled I-210 freeway and generally downwind of Los Angeles, therefore affected by motor vehicle emissions (Atkinson et al., 1988). Samples were collected by means of hi-vol samplers equipped with Teflon-impregnated glass fiber filters followed by PUF plugs. Table 4-3 shows the average (from three daytime and three nighttime samples) concentrations of PAH measured and the ratios of their concentrations to that of BeP. Unfortunately, no alkylated phenanthrenes were measured.

As can be seen from the comparison of Tables 4-2 and 4-3, the concentrations of all PAH measured in Glendora were much lower than those measured in the Tunnel, as would be expected. However, the ratios of the concentrations of particle-bound PAH to that of BeP were also different for the two sites, usually much lower for the Glendora site (with the exception of higher molecular weight PAH, indeno[1,2,3-cd]pyrene, benzo(ghi)perylene, and coronene). This may indicate either contributions from sources other than motor vehicles in the Glendora study or PAH photochemical transformations occurring on particles prior to or during hi-vol sample collections (or both). The generally higher PAH concentrations during the nighttime sampling periods than during the daytime sampling periods (Atkinson et al., 1988) seem to support the latter possibility (however, the influence of meteorology cannot be excluded). This conclusion is also consistent with high levels of photochemical pollutants observed in Glendora; for example the daily maxima of ozone concentrations (which occurred always between 2 and 5 pm) ranged from 160 to 240 ppb throughout the entire nine days of the study.

Table 4-3

Average Ambient Concentrations of PAH Measured in Glendora, CA[a]

PAH	Total Concn., ng/m^3 (particle concn.)	Ratio to BeP
Phenanthrene[b]	20	-
Anthracene[b]	1.0	-
Fluoranthene	5.6 (0.26)[c]	0.27
Pyrene	4.1 (0.35)[c]	0.37
Benzo(ghi)-fluoranthene	0.26	0.28
Cyclopenta(cd)-pyrene	0.09	0.1
Benz(a)anthracene	0.2	0.22
Chrysene/Tri-phenylene	1.0	1.1
Benzofluoranthenes [b,j + k]	1.6	1.7
Benzo(e)pyrene	0.94	1
Benzo(a)pyrene	0.33	0.35
Indeno[1,2,3-cd]-pyrene	1.6	1.7
Benzo(ghi)perylene	3.8	4.0
Coronene	2.8	3.0

[a] From Atkinson et al., 1988.
[b] Phenanthrene and anthracene were not present on filters, only on PUF plugs.
[c] Fluoranthene and pyrene are distributed between gas and particulate phases; numbers in parentheses represent particle concentrations.

4.3 Nitroarene Concentrations in Ambient Air

As discussed in Section 2.2.3, diesel particulate matter contains a variety of nitroarenes, with 1-nitropyrene being the most abundant among identified nitro-PAH. The concentration of 1-nitropyrene was measured in the extract of particulate samples collected at the Allegheny Mountain Tunnel on the Pennsylvania Turnpike (Gorse et al., 1983). This concentration was 2.1 ppm and ≤ 5 ppm (by mass) of the extractable material from diesel and spark-ignition vehicle particulate matter, respectively. These values are much lower than would be predicted on the basis of laboratory dilution tunnel measurements either for diesel or for spark-ignition engines. Unfortunately, there are no published data on other than 1-nitropyrene nitroarene concentrations in tunnels or roadsides.

Several nitroarene measurements were conducted in airsheds heavy impacted by motor vehicle emissions (Arey et al., 1987; Atkinson et al., 1988; Zielinska et al., 1989a, 1989b; Ciccioli et al., 1989). For example, ambient particulate matter samples were collected at three sites (Claremont, Torrance, and Glendora) situated in the Los Angeles Basin, with the Claremont and Glendora sites being ~30 km and ~ 20 km, respectively, northeast and the Torrance site ~20 km southwest of downtown Los Angeles (Arey et al., 1987; Atkinson et al., 1988; Zielinska et al, 1989a, 1989b). The sampling was conducted during two summertime periods (Claremont, September 1985 and Glendora, August 1986) and one wintertime period (Torrance, January-February 1986). Table 4-4 lists the maximum concentrations of nitropyrene and nitrofluoranthene isomers observed at these three sites during the daytime and nighttime sampling periods.

As can be seen from this table, 1-nitropyrene (1-NP), the most abundant nitroarene emitted from diesel engines, is not the most abundant nitroarene observed in ambient particulate matter collected at three sites heavy impacted

Table 4-4

The Maximum Concentrations of Nitrofluoranthene (NF) and Nitropyrene (NP) Isomers Observed at Three South Coast Air Basin Sampling Sites

Nitroarene, Collection Period	Concentration, pg/m^3, at Claremont[a,b]	Glendora[c,d]	Torrance[a,e]
2-NF, day	40	350	410
2-NF, night	1,700	2,000	750
3-NF, day	3	ND[f]	~3
3-NF, night	~3	ND	70
8-NF, day	2	3	8
8-NF, night	2	4	50
1-NP, day	3	15	60
1-NP, night	10	15	50
2-NP, day	1	14	50
2-NP, night	8	32	60

[a] From Zielinska et al., 1989b.
[b] Daytime sample collected from 1200 to 1800 hr and nighttime sample from 1800 to 2400 hr on 9/13/85.
[c] From Atkinson et al., 1988.
[d] Daytime sample collected from 0800 to 2000 hr on 8/20/86 and nighttime sample from 2000 to 0800 hr on 8/20-21/86.
[e] Daytime sample collected from 0500 to 1700 hr on 1/28/86 and nighttime sample from 1700 to 0500 hr on 1/27-28/86.
[f] ND: none detected.

by motor vehicle emissions. Of the two nitropyrene isomers present, 2-nitropyrene (2-NP), the main nitropyrene isomer formed from the gas-phase OH radical initiated reaction with pyrene (see Section 3.2.1), is sometimes more abundant. 2-Nitrofluoranthene (2-NF) was always the most abundant nitroarene observed in ambient particulate matter collected at these three sites and this nitrofluoranthene isomer is not present in diesel and gasoline vehicle emissions (see Table 2-7, Section 2.2.3, and, for example, Ciccioli et al., 1989). 2-Nitrofluoranthene is the only nitroarene produced from the gas-phase OH radical initiated and N_2O_5 reactions with fluoranthene (see Sections 3.2.1 and 3.2.2), whereas mainly 3-nitrofluoranthene, followed by lesser amounts of 1-, 7-, and 8-nitroisomers are present in diesel particulate matter and are produced from the electrophilic nitration reactions of fluoranthene.

Figure 4-1 shows the comparison of the nitroarenes formed from the OH radical-initiated reaction of fluoranthene and pyrene in an environmental chamber (upper trace) with the ambient samples collected at Torrance (lower trace) [from Arey et al., 1989]. The presence of N_2O_5 during the nighttime winter collections in Torrance was very unlikely, given the high level of NO present at sunset. More likely a relatively high level of OH radicals was present due to the measured high concentration of HNO_2, which photolyzes to yield OH radicals. This suggests that all isomers observed in Figure 4-1 (lower trace), with the exception of 1-nitropyrene, are the product of the OH radical-initiated reactions of the parent PAH. Direct emissions may account for the 1-nitropyrene (and 3-nitrofluoranthene) observed at relatively low levels in these ambient samples (see Zielinska et al., 1989b, for full discussion of all the mw 247 nitroarenes observed in ambient particles).

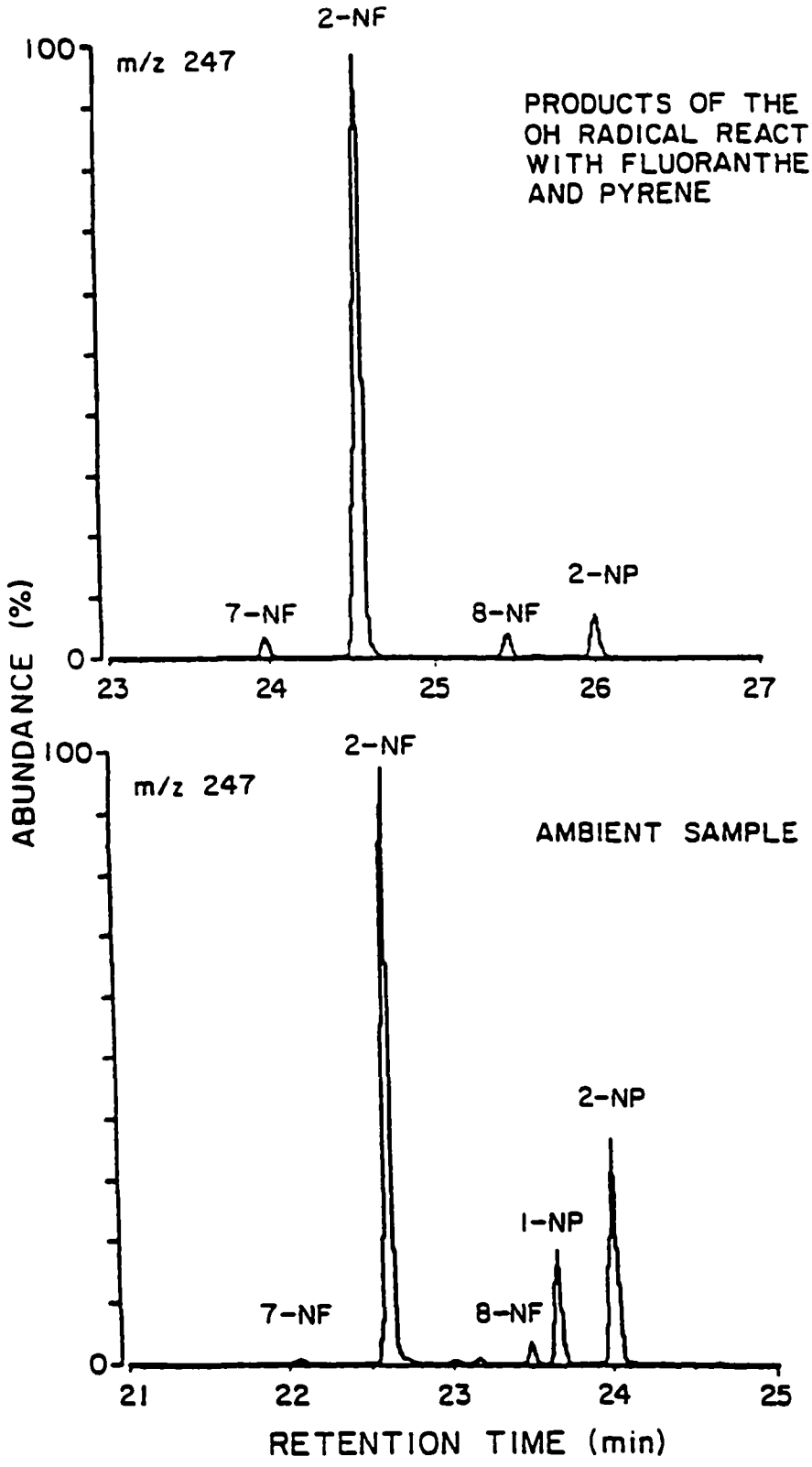

Figure 4-1. Mass chromatograms of the molecular ion of the nitrofluoranthenes (NF) and nitropyrenes (NP) formed from the gas-phase reaction of fluoranthene and pyrene with the OH radicals (top) and present in ambient particulate sample collected at Torrance, CA (bottom)

Although the reaction with OH radicals is the major atmospheric loss process for gas-phase fluoranthene and pyrene (see Table 3-1, Section 3.2.1), evidence for atmospheric formation of 2-nitrofluoranthene from N_2O_5 reaction with fluoranthene has also been reported (Zielinska et al., 1989b). Since the 2-nitrofluoranthene/2-nitropyrene yield ratio for N_2O_5 reactions, observed from environmental chamber experiments, is > 100, compared to ~10 for the OH radical reaction (see Table 3-2, Section 3.2.1), the high 2-nitrofluoranthene/2-nitropyrene concentration ratio in ambient samples suggests a contribution from the N_2O_5 reaction with fluoranthene. Figure 4-2 shows a comparison of a wintertime sample collected in Torrance (upper trace) with a summertime sample collected in Claremont (lower trace). The 2-nitrofluoranthene/2-nitropyrene ratio reached ~ 200 for the summer night sample. The N_2O_5 concentration was calculated to be ~ 5 ppb for this night, which supports the suggested formation route of 2-nitrofluoranthene via reaction with N_2O_5 (Zielinska et al., 1989b).

The evidence presented above, as well as the observation that 2-nitrofluoranthene has been the most abundant mw 247 nitroarene in ambient samples collected worldwide (Ramdahl et al., 1986), strongly suggests that the atmospheric formation from the parent PAH, not the direct automotive emissions, is the major source of these nitroarenes in ambient air. However, under certain sampling conditions, when ambient particulate matter is collected very close to emission sources, the mw 247 nitroarene profile may be different. For example, in urban samples collected during wintertime rush hours at a central square of Rome, Italy, at a height of 1.5 m above street level, 2-nitrofluoranthene and 2-nitropyrene were not observed (Cicciolli et al., 1989).

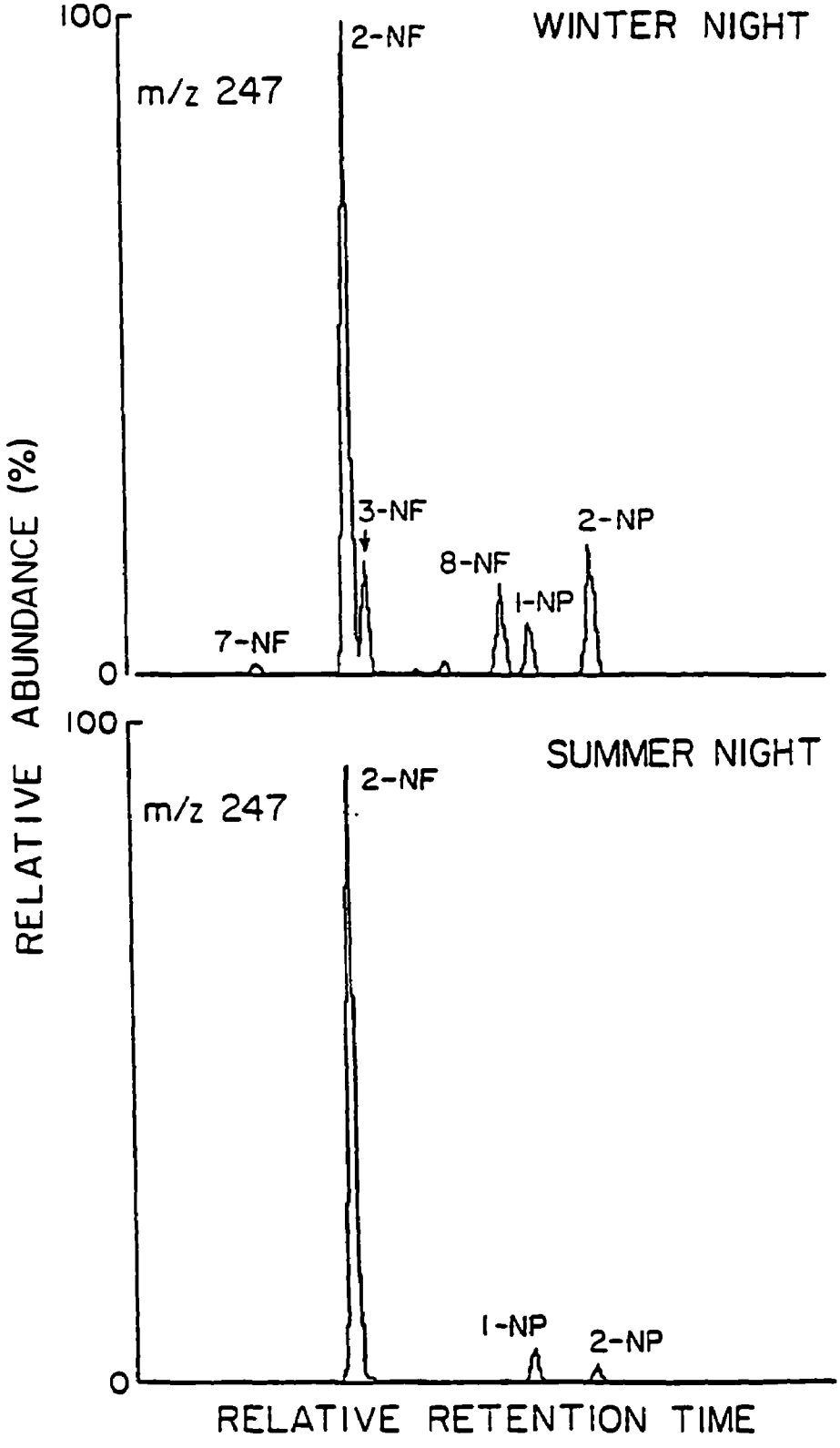

Figure 4-2. Mass chromatograms of the molecular ion of the nitrofluoranthenes (NF) and nitropyrenes (NP) present in ambient particulate samples collected in Torrance, CA (top) and Claremont, CA (bottom)

4.4 The Need for Atmospheric Tracers of Diesel Emissions

Receptor source-apportionment models assist in the identification of the principal sources of airborne pollutants and in the determination of source contributions to ambient aerosol mass concentrations (or gas- or particulate-phase species concentrations, light extinction, etc.). The technique ideally used would require the measurement at receptor sites of selected "tracers," i.e., constituents which can be attributed to specific emission sources. Trace elements and particle mass are most widely used as tracers. However, emissions from certain sources are difficult to distinguish by this means. For example, potassium, which is widely used as a wood smoke tracer, is also abundant in resuspended soil and cigarette smoke. Due to increasing use of unleaded gasoline, the ambient concentrations of traditional motor vehicle tracers, Pb and Br, are diminishing and there is a need to identify alternative tracers for mobile sources. In addition, some sources of toxic air pollutants do not emit trace metals.

Unique tracers, which could be used to distinguish diesel emissions from those of spark-ignition engines, have not yet been identified in diesel exhausts. However, as has been demonstrated by an experiment conducted in Vienna, Austria (Horvath et al., 1988), such tracers can be added deliberately. In the Vienna case, the rare-earth element dysprosium was added in the form of an organo-metallic compound to the entire diesel fuel supply in Vienna and the vicinity. By measuring the amount of this tracer in the atmospheric samples, the contribution of diesel vehicle emissions to the particulate pollutants in Vienna was determined (it ranged from ~ 12 to 33%). This approach, however, is costly and not always practical.

A second, much more approximate approach used in the past for estimating diesel exhaust contributions to ambient aerosol was based on the fact that diesel- and gasoline-fueled vehicles coexist on the highways. A "highway aerosol signature" was constructed as an emissions-weighted average of the chemical composition of the aerosol from gasoline-powered automobiles and trucks, diesel-powered automobiles and trucks, tire dust, and brake dust (Cass and MacRae, 1983). The lead content of the leaded gasoline burned by the gasoline-powered vehicles was used to determine the amount of "highway" aerosol in the ambient air. The diesel exhaust contribution was estimated in proportion to the relative contribution of diesel exhaust in the highway emission profile (which was based on source test data). However, as mentioned above, the lead tracer approach will not be possible in the near future, as lead is being removed from gasoline. In addition, this method does not address the emissions from diesel engines used in railroad locomotives, ships, off-highway construction equipment, etc.

Finally, it has been suggested that, since diesel particulate emissions are enriched in elemental carbon (EC), the elemental carbon content of an ambient particle, when scaled in proportion to the EC content of diesel exhaust, places an upper limit on the amount of diesel exhaust aerosol that can be present in an ambient sample (Cass, 1990). However, the reported values for elemental carbon in diesel exhausts range from 10 to 90% (see, for example, Williams et al., 1989b), although in most cases they are roughly 70% (see Table 2-3, Section 2.2.1). In addition, other combustion sources may contribute to the EC content of ambient particles.

Since organic compounds are emitted from all combustion sources, the potential exists for their use in receptor modeling. Reviews of existing literature data (see, for example, Daisey et al., 1986) revealed the potential

usefulness of certain classes of organic compounds in developing "fingerprints" for specific emission sources. At the same time, it is clear that the existing source emission data are not sufficient for receptor modeling purposes, and much more experimental data are required to develop consistent emission profiles for specific emission sources.

The class of organic compounds most suitable for serving as a source tracer should be:

- emitted in relatively high concentration, to allow small sample sizes and short sampling times;
- relatively easy to separate from other classes of organic compounds;
- relatively easy to identify and quantify on the basis of the chromatographic and spectral properties of its members;
- chemically stable to assure the same composition in source and receptor sites, i.e., it should not undergo atmospheric transformations.

In addition, the composition pattern (fingerprint) for this class should differ between different emission sources to assist in distinguishing among them.

Polycyclic aromatic hydrocarbons were advocated as potential tracers of various types of combustion (Daisy et al., 1986). PAH are present in all combustion sources and their relative proportions in emissions from a given source type frequently vary over several orders of magnitude. In addition, good sampling and analytical methods already exist for this class of compounds. However, although the PAH concentrations in motor vehicle emissions were frequently measured in the past, the purpose of these measurements has been to determine an emission rate under a given set of conditions rather than to establish a PAH "fingerprint" for receptor source modeling. Therefore there is an apparent lack of compatibility among available motor vehicle PAH emission profiles.

At the present time, it is not possible to make any firm judgment as to the possibilities for distinguishing diesel emissions from those of spark-ignition engines. However, some PAH compounds appear to be promising. As mentioned in Section 2.2.3 of this Chapter, diesel exhaust is enriched in alkyl-substituted PAH, particularly alkyl-phenanthrenes, relative to parent compounds. It is known that low-temperature formation (petrogenesis) of PAH produces a mixture enriched in alkyl-substituted PAH and other kinetically favored compounds, whereas high-temperature processes (combustion and pyrolysis) favor the generation of unsubstituted compounds. In agreement with this, it has been reported that the total concentration of alkyl-PAH in diesel emissions increased, as the cylinder exhaust temperature decreased (Jensen and Hites, 1983).

Benner and co-workers (1989) reported that the high concentrations of methyl- and dimethylphenanthrenes measured in the Baltimore Harbor Tunnel suggest the contribution of diesel emission sources in the Tunnel (see also Section 4.2 of this Chapter). However, alkyl substituted PAH are also abundant in coal and coal-derived material (see, for example, White, 1983) and shale oil (Garrigues et al., 1987). More data on alkylated PAH concentrations in different combustion sources are clearly needed.

The most important limitation for PAH being used as emission markers is their relatively high chemical reactivity (see Section 4 of this Chapter). Thus, the PAH profile determined at the emission source may differ considerably from the source PAH profile as it exists in the ambient atmosphere. However, it has been suggested (Miguel and Pereira, 1989) that some presumably more stable particle-bound PAH, such as benzo(k)fluoranthene, benzo(ghi)perylene, and ideno(1,2,3-cd)pyrene can be used as tracers of automotive emissions at receptor sites which have no other major sources of PAH.

Taking chemical reactivity and ambient abundance into account, alkanes seem to be more suitable for tracing motor vehicle emissions than PAH. The lower homologs which exist entirely in the gas-phase react slowly with the OH radicals and their atmospheric lifetimes are on the order of several days (see Table 3-1, Section 3.2.1). The higher, mostly particle-associated homologs (C > 20) are relatively unreactive. The n-alkanes originating from natural sources (e.g., plant waxes) could be distinguished from those originating from anthropogenic sources on the basis of the odd-to-even carbon number preference. Also, the ratio of normal to branched isomers is lower in emissions from fossil fuel combustion than for biogenetically derived alkane aerosol (Simoneit, 1984). In addition, the literature data indicate that a homologous series of alkylcyclohexanes (C_nH_{2n} with n ranging from n = 16 to 29) are characteristic for spark-ignition vehicle emissions, but are only trace components of diesel exhaust (Simoneit, 1984). It has been also suggested that it may be possible to distinguish emissions from diesel and spark-ignition engines based on the ratios of methylated isomers to total branched isomers (Boone et al., 1987).

However, the very limited data sets available for source emissions indicate that the relative concentrations of the particulate C-24 to C-36 alkanes vary only one order of magnitude among different source types. It is possible that the inclusion of the semivolatile alkanes would extend the range of relative concentrations. Clearly, much more measurement of alkanes in source emissions is needed to allow comparison among sources.

In summary, the existing data indicate that it may be possible to use organic compound profiles, perhaps in combination with trace element tracers, to assist in distinguishing among diesel and other particulate pollutant sources. However, the determination of organic and inorganic compositions of emissions

from a number of sources, using sampling and analytical methods appropriate for the purpose of receptor source apportionment modeling, is clearly necessary.

5.0 MUTAGENICITY OF RESPIRABLE AMBIENT PARTICLES

In 1976, Tokiwa and co-workers reported that organic extracts of ambient particles collected in Japan were active in the Ames *Salmonella typhimurium* assay, when tested in the presence of homogenized rat liver tissue, colloquially known as S9 mix (Tokiwa et al., 1976; 1977). This was not surprising, since it was known then that ambient particulate matter contained promutagenic PAH. Soon thereafter, a direct mutagenic activity (i.e., in the absence of S9 mix) of extracts of ambient particles collected in major cities throughout the world was reported (see, for example, Pitts et al., 1977; Talcott and Wei, 1977; Tokiwa et al., 1980; Lofroth, 1981; Finlayson-Pitts and Pitts, 1986, and references therein). It has been shown that this direct activity was primarily associated with organic species present in respirable particles of < 2 μm diameter.

Reports of the presence of direct-acting mutagenic species in extracts of ambient particulate organic matter (POM) and vehicle emissions (see previous chapter) resulted in extensive worldwide investigations, which ultimately resulted in the identification of a number of PAH derivatives, mostly in diesel particulate extracts (see, for example, Schuetzle, 1983). The use of short-term bacterial bioassay in conjunction with analytical procedures, so-called "bioassay directed chemical analysis," proved to be extremely useful in identification of the key biologically active compounds in complex environmental mixtures (see Schuetzle and Lewtas, 1986, for a recent review). This technique is briefly described in Section 5.1 below.

The contribution of mutagenic species present in diesel particulate extracts to the mutagenic activities of ambient particulate extracts has been the subject of several studies (see, for example, Gibson, 1983; Tokiwa et al., 1983; Siak et al., 1985). The contribution of atmospherically formed nitroarenes to

the mutagenic activities of particulate samples collected in southern California has been also recently assessed (Arey et al., 1988b). These studies are briefly discussed in Section 5.2 below.

5.1 Bioassay Directed Chemical Analysis

Most environmental samples are complex mixtures and comprise thousands of chemical compounds. Identification of the biologically active compounds, often present in minute quantities, by traditional analytical methods, would present an enormous if not impossible task. It became apparent in the late 1970s that the short-term bioassays could be used in combination with chemical fractionation to simplify greatly the process of identifying significant mutagens in complex environmental samples, such as diesel or ambient particulate extracts (Schuetzle and Lewtas, 1986).

The Ames *Salmonella* bacterial strains, used with and without S9 mix, provide information about the general classes of chemicals causing mutagenic response (e.g., frameshift versus base pair substitutions, promutagen or direct-acting mutagen). More recently, tester strains that are sensitive to certain classes of compounds have been developed. For example, strain TA98NR, developed by Rosenkranz and co-workers (see, for example, McCoy et al., 1981), is deficient in nitroreductase enzymes and therefore gives a reduced response to nitro-PAH. Strain TA98 in conjunction with strain TA98NR are most frequently used for assays of environmental samples (see Rosenkranz and Mermelstein, 1983, for more detailed discussion).

Figure 5-1 illustrates the principles of bioassay-directed chemical analysis (from Schuetzle and Lewtas, 1986). Proper sampling, storage, and

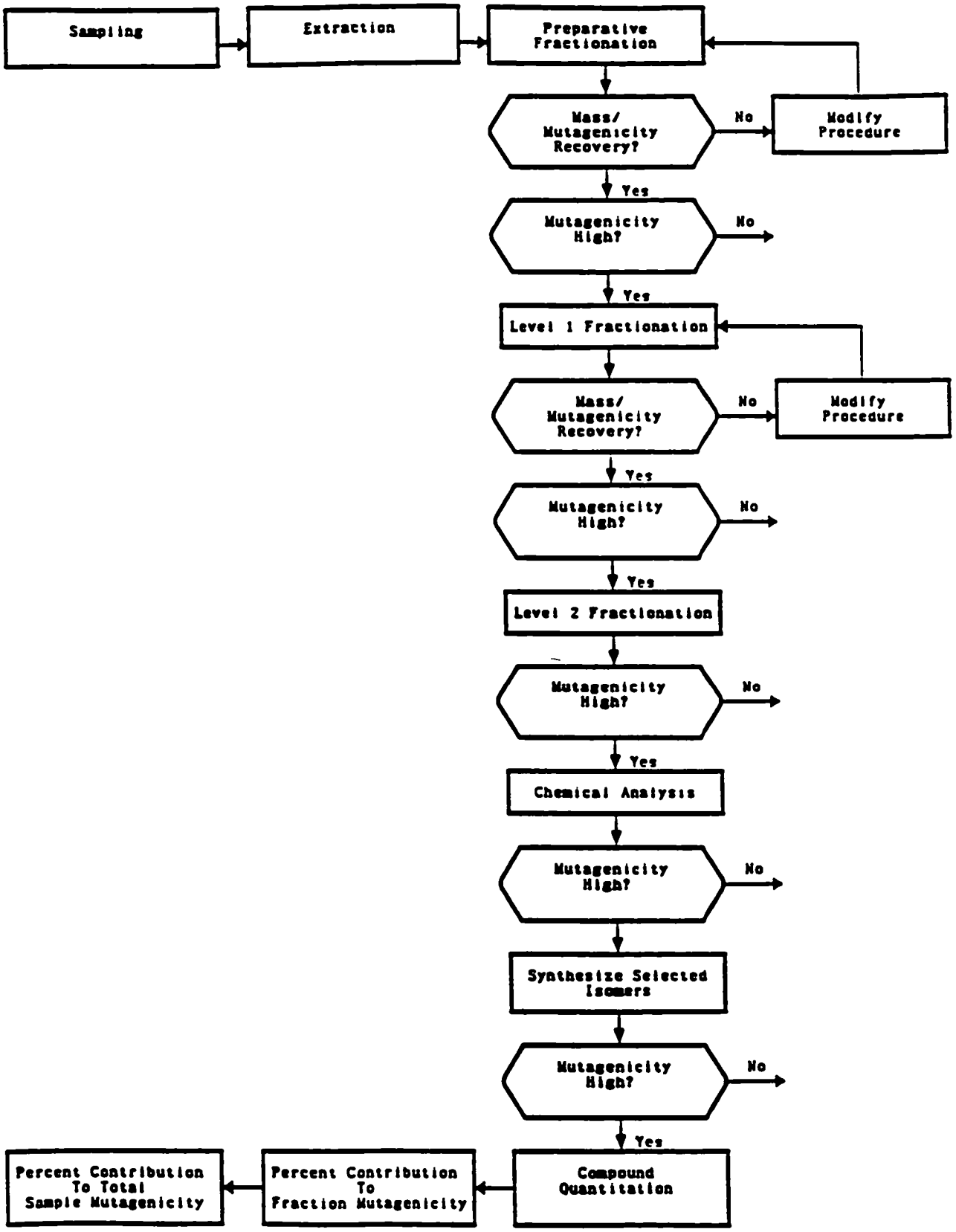

Figure 5-1. Protocol for bioassay-directed chemical analysis (from Schuetzle and Lewtas, 1986).

extraction of environmental samples are crucial parts of the analysis. Sequential extraction with increasingly polar solvents or binary solvents is most frequently used to separate organic material from particles (see Section 2.2.3). The following step, preparative fractionation, achieves crude separation of extract into several less complicated fractions. The two most widely used prefractionation techniques are chromatography on an open silica column to separate groups of compounds on the basis of polarity and separation of compounds into acidic, basic, and neutral fractions (Schuetzle and Lewtas, 1986). These fractions contain hundreds of compounds and are still too complicated to be characterized chemically. However, the bioassay analysis is used to decide which fractions should be analyzed further.

Normal-phase high performance liquid chromatography (HPLC) is usually employed as a level 1 fractionation step (see Figure 5-1). The reference compounds 1-nitronaphthalene and 1,6-pyrenequinone were proposed (see Section 2.2.3) as chemical markers to designate the separation of samples into nonpolar, moderately polar, and polar fractions. It was found that the nonpolar fractions accounted for less than 2-3% of the total extract mutagenicity and that the distribution of mutagenicity between moderately polar and polar fractions was dependent on the sample origin (Schuetzle et al., 1985).

Although a multitude of compounds were identified or tentatively identified from the chemical analysis of fractions from level 1 fractionation (see Section 2.2.3), it soon became obvious that these fractions were still too complex to allow identification of many less abundant chemical mutagens. Further separation of each fraction into subfractions using level 2 (see Figure 5-1) fractionation was necessary. This fractionation is usually achieved by employing reversed-

phase HPLC or normal-phase HPLC with a different solvent system and/or chromatographic column than that used in level 1 fractionation.

The analytical techniques most frequently used for the characterization of HPLC fractions include high resolution capillary column gas chromatography (GC) with selective detectors and/or coupled with mass spectrometry (GC/MS). Comparison of the results from different mass spectrometric ionization techniques (i.e., electron impact versus chemical ionization) may be helpful in the identification of individual compounds. However, very polar or labile compounds cannot be analyzed with GC/MS techniques due to losses resulting from adsorption, thermal decomposition and chemical interactions occurring on GC columns. Direct-insertion probe coupled with high-resolution MS or MS/MS techniques have been used as screening techniques for tentative identification of polar compounds. One of the most promising analytical techniques for the analysis of polar PAH appears to be supercritical fluid chromatography and HPLC coupled with mass spectrometry (see, for example, Schuetzle et al., 1985, for more detailed discussion of new analytical techniques for the identification of polar mutagens).

5.2 Contribution of Nitroarenes to Ambient Mutagenicity

The distribution of direct-acting mutagenicity between the moderately polar (MP) and polar (P) fractions for particulate samples of different origins is shown in Figure 5-2 (from Schuetzle et al., 1985). As mentioned above, the nonpolar fractions account for less than 2-3% of the total mutagenicity of extracts; thus, they are not shown in this figure.

According to Figure 5-2, for light-duty diesel particulate extracts, most of the direct-acting mutagenicity (65-75%) is associated with the moderately

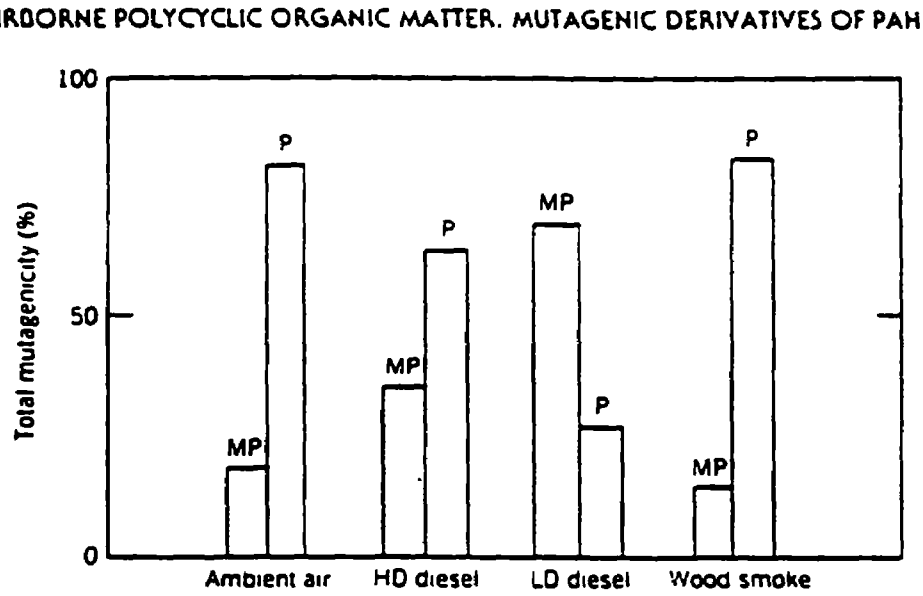

Figure 5-2. Distribution of direct-acting mutagenicity (TA98, -S9) between moderately polar (MP) and polar (P) fractions of extracts of particulate matter in ambient air, wood smoke, and in the exhaust from heavy duty (HD) and light duty (LD) motor vehicles. Adapted from Schuetzle et al. (1985).

polar fraction. In contrast, the polar fractions of extracts of ambient particles, heavy-duty diesel particles, and wood smoke particles are said to contain more than 65% of the total extract mutagenicity. As mentioned above (see also, Section 2.2.3), the compounds responsible for the mutagenicity of these polar fractions have not been yet identified.

Up to 40% of the direct-acting mutagenicity of total extracts of light-duty diesel particles can be accounted for by six nitroarenes (1-nitropyrene, 3- and 8-nitrofluoranthene, and 1,3-, 1,6-, and 1,8-dinitropyrene), eluting in the moderately polar fraction (Salmeen et al., 1984). In contrast, these nitroarenes accounted for not more than 3% of the total mutagenic activity of ambient particulate samples collected at urban and suburban sites (Siak et al., 1985).

The contribution of atmospherically formed nitroarenes, 2-nitrofluoranthene, and 2-nitropyrene (see Section 3.2), to the mutagenicity of ambient particulate samples collected in southern California has been recently assessed (Arey et al., 1988b). This contribution could be compared to that of 1-nitropyrene and 3- and 8-nitrofluoranthene, regarded as direct emissions from various combustion sources.

Table 5-1 gives the mutagen density (revertants/m^3) of eight ambient samples along with the calculated percent contributions of the measured nitroarenes to this mutagenicity. The samples were collected in Torrance, CA, during wintertime and in Claremont, CA, during summertime (see Section 4.4 for more detailed description of sampling sites and maximum nitroarene concentrations).

Although 2-nitrofluoranthene was always the most abundant nitroarene measured in these ambient samples, the high mutagenic activity of 8-nitrofluoranthene resulted, in one instance, in this much less abundant isomer

Table 5-1

Contribution of Nitrofluoranthene (NF) Isomers to the Direct Mutagenicity of Ambient Particulate Extracts[a]

Collection Date and Location	Collection Time (h)	Mutagen Density[b] (rev m^{-3})	% Contribution to Mutagenicity					
			2-NF	3-NF	8-NF	1-NP	2-NP	Total
1/27-28/86 Torrance, CA	1700-0500	120	2.6	1.8	3.1	0.1	0.8	8.4
1/28/86 Torrance, CA	0500-1700	120	1 4	-[c]	0.5	0.1	0.7	2.7
2/24-25/86 Torrance, CA	1800-0600	34	3 9	-	-	0 2	1 4	5 5
2/25/86 Torrance, CA	0600-1800	73	1 6	-	-	0 1	0.9	2.6
9/14/85 Claremont, CA	0600-1200	35	1.0	-	-	0.2	0.1	1.3
9/14/85 Claremont, CA	1200-1800	15	0.8	-	-	0.2	0.1	1.1
9/14/85 Claremont, CA	1800-2400	40	5.2	0.4	0.4	0.2	0.2	6.4
9/15/85 Claremont, CA	0000-0600	20	2.1	-	0.3	0.1	0.2	2.7

[a]From Arey et al., 1988b.
[b]Tested on strain TA98(-S9).
[c]Not quantified or only an upper limit determined.

contributing a greater fraction of the ambient activity than did 2-nitrofluoranthene. In all remaining cases, the contribution of 2-nitrofluoranthene to the ambient mutagenicity was higher than that of the other measured nitroarenes and ranged from ~1% to ~5%. In contrast, 1-nitropyrene never contributed more than 0.2%.

Thus, although nitroarenes directly emitted from combustion sources may, in some cases, contribute significantly to ambient mutagenicity (see, for example Gibson, 1983), the contribution of atmospherically formed nitroarenes should be also recognized.

6.0 SUMMARY

Major research programs were carried out in the late 1970s and early 1980s to understand the physical and chemical characteristics of emissions from diesel engines and the biological effects of these emissions. Although new control technologies are being introduced into currently manufactured diesel vehicles, the effect of these changes on diesel emissions is likely to be visible in the future. At the present time, diesel vehicles manufactured in the late 1970s and early 1980s are still on the road and, in this sense, data collected from that time period are still valid.

However, many of these data were collected using laboratory dynamometers with selected new, or at least "well-tuned" to manufacturers specifications, vehicles. The well-controlled conditions of the dynamometer tests have many benefits but do not necessarily represent vehicle emissions under real on-road conditions. The small number of vehicles tested in the laboratory is not truly representative of the distribution within the on-road vehicle fleet. Although several roadway and tunnel emission measurements were performed in the past, the data base on mobile sources emission rates, necessary to assess the role of vehicle emissions in air pollution problems, is still not sufficient. More measurements carried out under realistic on-road conditions are necessary, in particular for gaseous- and particulate-phase organic compounds present in vehicle emissions.

Once released into the atmosphere, diesel emissions are subjected to dispersion and transport and, at the same time, to chemical and physical transformations into secondary pollutants, which may be more harmful than their precursors. Thus, a knowledge of diesel emissions at or near their sources is no longer sufficient to assess fully the impact of these emissions on human

health and welfare. The understanding of physical and chemical changes that primary diesel emissions undergo during their transport through the atmosphere is equally important. As a result of the last two decades of laboratory and ambient experiments and computer modeling, a comprehensive set of data now exist concerning the atmospheric loss processes and transformation of automotive emissions. However, our knowledge concerning the products of these chemical transformations is still very limited. Study is required to determine the products from the OH radical-initiated reactions of the aromatic and aliphatic hydrocarbons, the major components of automobile emissions. The atmospheric transformation products of PAH and their oxygen-, sulfur-, and nitrogen-containing analogs require study in the gaseous and adsorbed phases. In particular, the reactions occurring in adsorbed phases on atmospherically relevant surfaces are poorly understood and require further study. In addition, gas-to-particle conversion processes and the chemical processes that lead to aerosol formation should be further investigated.

The quantitation of the contribution of diesel emissions to total ambient aerosol mass concentration is not possible without developing a specific profile for diesel emissions, a "fingerprint" which may be used in receptor source apportionment models. The existing data indicate that it may be possible to use PAH and/or alkylated PAH, alkanes, and possibly certain unique compounds to assist in distinguishing between diesel and other pollutant sources. However, the available data are not adequate for use in receptor modeling and study is required to determine the profile of diesel emissions, using sampling and analytical methods appropriate to the purpose of receptor modeling.

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4. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

The objective of this chapter is to evaluate the potential noncarinogenic health effects of diesel exhaust. Data pertaining to exposures to whole diesel exhaust will be presented first, followed by a comparison of the effects of filtered and unfiltered exhaust. Filtered exhaust consists of the gaseous components of the exhaust without the associated particulate matter.

4.1. HEALTH EFFECTS OF WHOLE EXHAUST

4.1.1. Human Data

4.1.1.1. Short-Term Exposures

Kahn et al. (1988) reported on the occurrence of 13 cases of acute overexposure to diesel exhaust. Twelve cases included symptoms of mucous membrane irritation, headache, and light-headedness. Eight victims reported nausea, four reported a sensation of unreality, four reported heartburn, three reported weakness, numbness and tingling in extremities, and vomiting, two reported chest tightness, and two others reported wheezing (no other data were given).

4.1.1.1.1. *Diesel exhaust odor*

Diesel exhaust odor is generally considered a nuisance pollutant; however, at high intensities it may produce physiological and psychological effects to warrant concern. Strong unpleasant odors may cause nausea, headache, loss of appetite, and psychological stress. The intensity of the odor is an exponential function of the concentration such that a tenfold change in the concentration of the odorants will alter the intensity of the odor by one unit. Diesel odor is measured in terms of a 12-step (D-units) odor intensity scale (Turk, 1967) or in terms of a 7-step Total Intensity of Aroma (TIA) scale (CRC, 1979; Levins, 1981).

Surveys have been undertaken to evaluate the general public's response to diesel exhaust odor. Hare and Springer (1971; Hare et al., 1974) found that at a D rating of about 2 (TIA = 0.9), about 90 percent of the participants perceived the odor and almost 60 percent found it objectionable. At a D rating of 3.2 (TIA = 1.2), about 95 percent perceived the odor and 75 percent objected to it, and at a D rating of 5 (TIA = 1.8) about 95 percent objected to it. Linnell and Scott (1962) evaluated the odor threshold for diesel exhaust and found that a dilution factor of 140 to 475 was needed to reduce the odor intensity to the threshold level.

4.1.1.1.2. *Pulmonary and respiratory effects*

Battigelli (1965) conducted tests on 13 human volunteers and found that 15-min to 1-h exposures to diesel exhaust did not have a significant effect on pulmonary resistance. The exhaust was generated from a single-cylinder, four-cycle, 7-hp engine (no other data were given). Pulmonary resistance was measured through the simultaneous recording of esophageal pressure and flow obtained through electrical differentiation of the volume signal from the spirometer of a plethysmograph. Three dilutions of exhaust were used. The concentration of the exhaust particles was not reported. The three exhaust dilutions contained on the average 1.3, 2.8, and 4.2 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. The two higher concentrations of exhaust were strongly irritating to the eyes within 10 min.

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 et al. (1978) found that both forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) decreased by 0.05 L in 60 diesel exhaust-exposed miners, an amount not substantially different from the reductions seen in miners not exposed to diesel exhaust (0.02 and 0.04 L, respectively). Miners with a history of smoking had more flow rate decrements over the shift than did nonsmokers. No information was given on pollutant concentrations.

Except for differences related to age, 120 underground iron ore miners exposed to diesel exhaust exhibited no significant workshift changes in FVC and FEV_1 when compared

with 120 matched controls (Jorgensen and Svensson, 1970). Both groups were subdivided with regard to smoking habit. Among the underground miners there was a higher frequency of bronchitis, but this was seen primarily in smokers and those in older age groups. Jorgensen and Svensson estimated that the miners could have been exposed to 0.5 to 1.5 ppm NO_2 and between 3 and 9 mg/m^3 of particulate matter, with 20 to 30 percent of the particles <5 μg. The majority of the particles were magnetic ore.

In tests on salt miners exposed to diesel exhaust, Gamble et al. (1978, as reported in Gamble et al., 1987a) found small changes in pulmonary function over the course of a workshift. These changes were not statistically significant, and they appeared to be related to the concentration of NO_2 (mean value 1.5 ppm) and not to the concentration of particulate matter (mean value 0.7 mg/m^3).

Ames et al. (1982) compared the pulmonary function of 60 coal miners exposed to diesel exhaust with a control group of 90 miners not exposed to exhaust. Measurements were made before and after an 8-h workshift. The workplace air concentrations of the primary exhaust pollutants were 2.0 mg/m^3 particulate matter (particle size not reported), 0.2 ppm NO_2, 12 ppm CO, and 0.3 ppm formaldehyde. In terms of FVC, FEV_1, and forced expiratory flow rate at 50 percent FVC (FEF_{50}) there were no significant differences between the two groups. However, coal miners exposed to diesel exhaust who were also smokers or ex-smokers did have significant reductions in FEV_1 and FEF_{50}. These reductions were similar to those seen in the nonexposed group who were also smokers.

In a study of 232 diesel bus garage workers, Gamble et al. (1987a) found some evidence of health effects associated with exposure to diesel exhaust. The prevalence of symptoms of cough, labored breathing, itching, burning and watering of eyes, chest tightness, and wheezing were higher (p <0.05) than in a comparison population of 248 battery workers. Comparisons were made without adjustment for age or smoking history. Eye irritation appeared to be the most sensitive indicator of exposure. There was no detectable association between the daily exposure and changes in pulmonary function. Mean reductions in FVC, FEV_1, peak flows, and flows at 50 percent and 75 percent FVC were not different from zero. Workers who had respiratory symptoms had slightly greater, but statistically insignificant reductions in FEV_1 and FEF_{50}. Although smokers had the greatest reductions

in pulmonary function over the workshift, these were not significantly different from those of nonsmokers or exsmokers. The concentration of particulate matter (particle size not reported) averaged 0.24 mg/m^3 (maximum mean value 0.61 mg/m^3). The concentration of NO_2 averaged <1.5 ppm.

Ulfvarson et al. (1987) evaluated workshift changes in pulmonary function in 15 bus garage workers, 25 crew members of two car ferries, and in 37 workers on roll-on roll-off ships. The latter group was exposed primarily to diesel exhaust, while the first two groups were exposed to both gasoline and diesel exhaust. In the diesel-exposed group, average 8-h exposures were 0.13 to 1.0 mg/m^3 of particulate matter, 0.02 to 0.8 mg/m^3 (0.016 to 0.64 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. FVC and FEV_1 were significantly reduced over the workshift ($p \leq 0.01$ and $p \leq 0.001$, 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 were not affected by the exposure to the diesel exhaust. Pulmonary function returned to normal after 3 d of nonexposure. No exposure-related correlation was found between the observed effects and concentrations of NO, NO_2, formaldehyde, or carbon monoxide; however, it was suggested that NO_2 adsorbed onto the exhaust particles may have contributed to the overall dose of NO_2 to the lungs. In a related study, in which six volunteers were exposed to diesel exhaust diluted to a level such that the particle concentration was 0.6 mg/m^3 and the NO_2 concentration was 3.8 mg/m^3 (2.0 ppm), there were no changes in pulmonary function. The exhaust was generated by a 6-cylinder, 2.38-L diesel vehicle operated at constant speed, equivalent to 60 km/h, and at about one-half full engine load.

4.1.1.2. Long-Term Exposures

Several epidemiological 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. When compared with a control population of comparable age and smoking history (154 railroad yard workers), no significant

differences were found in the exposed workers (statistical data not given). The frequency of symptoms of respiratory distress (i.e., dyspnea, cough, sputum) was not significantly different between the two groups; however, in both groups the majority of complaints came from smokers. For the exposed group, maximum workplace concentrations of air pollutants 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 particulate matter was not reported.

Reger et al. (1982) reported a higher incidence of symptoms of cough and phlegm, fewer symptoms of moderate to severe dyspnea, and generally lower pulmonary function (lower FVC, FEV_1, and flow rates at lower lung volumes) in underground coal miners exposed to diesel exhaust than in a group of matched controls (coal miners not exposed to diesel exhaust). However, there was no significant trend between health characteristics and years of exposure. When analyzed by age and smoking status, the data showed no consistent interacting effects. Mean concentrations of NO_x at the six mines ranged from 0 to 0.6 ppm NO_x for short-term area samples, 0.13 to 0.28 ppm for full shift personal samples, and 0.03 to 0.80 ppm for full shift area samples. Concentrations of particulate matter averaged 0.93 to 2.73 mg/m^3 for personal samples and 0 to 16.1 mg/m^3 for area samples. Five hundred and fifty underground miners and 273 surface workers were included in the study.

Attfield et al. (1982) evaluated 630 potash miners who were exposed to diesel exhaust and found a higher prevalence of symptoms of cough and phelgm, but no differences in pulmonary function (FVC and FEV_1) when compared with the predicted values derived from a logistics model based on data from blue collar workers not exposed to dust. Symptom prevalence was higher for smokers than nonsmokers. Environmental monitoring at the six mines indicated that NO_2 concentrations ranged from 0.1 to 3.3 ppm, aldehyde concentrations from 0.1 to 4.0 ppm, carbon monoxide from 5 to 9 ppm, and total dust concentrations from 9 to 23 mg/m^3. The ratio of total to respirable dust ranged from 2 to 11.

Gamble et al. (1983) evaluated symptoms of respiratory stress and pulmonary function in 259 salt miners exposed to diesel exhaust. After adjustment for age and smoking, the salt miners showed no increased prevalence of cough, phelgm, dyspnea, or airway obstruction when compared with coal miners, potash miners, and blue collar nonminers.

Mean predicted lung function values for the salt miners were lower than all comparison populations. Reductions of 2 to 4 percent in FEV_1 and FVC, 7 to 13 percent in FEF_{50}, and 18 to 22 percent in FEF_{75} were reported. These reductions were statistically significant ($p < 0.05$) except for FVC in blue collar workers. There was, however, no association between predicted pulmonary function and estimated cumulative exposure to particulate matter or estimated cumulative exposure to NO_2. 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 to 2.5 ppm. In a continuation of these studies, Gamble and Jones (1983) assessed the effect of exposure level on prevalence of respiratory symptoms and pulmonary function. The workers were grouped into low, intermediate, and high exposure categories; the average concentration of particulate matter being 0.40, 0.64, and 0.82 mg/m^3. respectively. A statistically significant association was found for the prevalence of phelgm in the 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 exposure. In a comparison with a control group of nonexposed workers, and after adjustment for age and smoking, the overall prevalence of cough and phelgm, but not dyspnea, was elevated. FEV_1 and FVC were within 4 percent of expected, which was considered to be within the normal range of variation for a nonexposed population.

In a study of 283 diesel bus garage workers, Gamble et al. (1987b) found some evidence of chronic respiratory health effects associated with exposure to diesel exhaust. When compared with a control population (716 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 a exposure-response trend but no apparent increase in prevalence. Mean pulmonary function was not reduced in the exposed population when compared with a control population, but reductions in FEV_1 of about 13 mL/yr for each year of employment were seen in workers with 10 or more years of tenure. There was a 2.5 percent incidence of pneumoconiosis in the exposed workers, but data were insufficient for further analysis.

Ames et al. (1984) conducted a 5-yr prospective study of 1118 underground coal miners to determine whether occupational exposure to diesel exhaust led to chronic respiratory effects. Changes in pulmonary function and the occurrence of symptoms of respiratory effects were evaluated in 280 miners exposed to diesel exhaust and in 838 control miners. Comparisons were controlled for age, years of underground mining, and for smoking status. In one group of 200 diesel-exposed miners from western states, forced expiratory flow rate at 50 percent FVC was significantly lower than that in the controls ($p < 0.001$); however, FVC, FEV_1, and chronic respiratory symptoms (i.e., chronic cough, chronic phlegm, and breathlessness) were not significantly different between the exposed and control groups. An internal analysis by cumulative years of exposure did not reveal any association between respiratory symptoms or pulmonary function parameters and exposure level.

In a preliminary study of 129 employees of a bus company, Edling and Axelson (1984) found a fourfold higher risk of mortality due to cardiovascular disease, even after adjusting for smoking history and allowing for at least 10 yr of exposure and 15 or more years of induction-latency time. However, In a later more comprehensive epidemiological study, Edling et al. (1987) evaluated mortality data covering a 32-yr period for a cohort of 694 bus garage workers and found no significant differences between observed and expected number of deaths due to cancer or cardiovascular disease. Information on exposure levels was not reported.

Purdham et al. (1987) evaluated respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations and in a control group of 11 onsite office workers. Twenty-four percent of the exposed group and 36 percent 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 logistics regression analysis. The frequency of respiratory symptoms was not significantly different between the two groups; however, baseline lung function measurements were significantly different. 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). Purdham et al. (1987) concluded that the lung function measurements provided evidence for an obstructive ventilatory defect. The stevedores were exposed to both diesel and gasoline exhaust. Concentrations of SO_2 were below detection

limits and only traces of NO_2 (0.5 ppm) were present in the work environment. Concentrations of CO exceeded 50 ppm for short periods of time. 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).

4.1.2. Animal Studies

4.1.2.1. Acute Exposures

The acute toxicity of undiluted diesel exhaust to rabbits, guinea pigs, and mice was assessed by Pattle et al. (1957). The engine used was a single-cylinder, air-cooled diesel engine with a rating of 3.75 to 6.0 (brake horsepower) at 1200 to 1800 rpm. Four different engine operating conditions were used, the exposures were for 5-h periods, and four rabbits, 10 guinea pigs, and 40 mice were tested under each exposure condition (no controls were used). Mortality was assessed up to 7 d 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 of particulate matter (particle size not reported), 0.056 percent CO, 23 ppm NO_2, and 16 ppm aldehydes. Exhaust containing 53 mg/m^3 of particulate matter, 0.038 percent CO, 43 ppm NO_2, and 6.4 ppm aldehydes resulted in low mortality rates (mostly below 10 percent) and moderate lung damage. Exhaust containing 122 mg/m^3 of particulate matter, 0.0418 percent CO, 51 ppm NO_2, and 6.0 ppm aldehydes produced high mortality rates (mostly above 50 percent) and severe lung damage. Exhaust containing 1070 mg/m^3 of particulate matter, 0.17 percent CO, 12 ppm NO_2, and 154 ppm aldehydes resulted in 100 percent mortality in all three species. High CO levels, which resulted in a carboxyhemoglobin value of 60 percent in mice and 50 percent in rabbits and guinea pigs, was considered to be the main cause of death in the latter case. High NO_2 levels were considered to be the cause of the 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.

4.1.2.2. Short-Term Exposures

EPA-Sponsored Studies. O'Neil et al. (1980) evaluated pulmonary function in nine mice exposed to light duty diesel exhaust for 3 mo (the exposure parameters, exhaust generating system, and concentrations of the exhaust components were not reported). When compared with five control mice, there were no significant changes in lung volume, vital capacity, residual volume, or diffusing capacity. However, the exposure did cause an accumulation of particles in alveolar macrophages and in the interstitium.

EPA conducted several short-term diesel exhaust toxicity studies using a 2.2-L Nissan CN6-33 engine coupled to a Chrysler Torque-Flite automatic transmission, Model A 727, and operated under a modified "California Cycle". In one study the effects of diesel exhaust exposures on the pulmonary function of cats was evaluated (Pepelko et al., 1980a). Eighteen male cats were exposed 20 h/d for 28 d. After a 14:1 dilution, the air in the exposure chambers contained an average of 6.4 mg/m^3 of particulate matter, 14.6 ppm CO, 2.13 ppm NO_2, 2.1 ppm SO_2, 0.577 mg/m^3 sulfates, and 31.56 ppm total hydrocarbons. With the exception of a decrease in maximum expiratory flow rate at 10 percent vital capacity, there were no significant changes in pulmonary function. Although there was no evidence of serious lung damage, a predominantly peribronchiolar localization of soot-laden macrophages within the alveoli resulted in focal pneumonitis or alveolitis.

In another EPA study the effects of a short-term diesel exhaust exposure on arterial blood gases, pH, blood buffering, body weight changes, lung volumes, and deflation PV curves of rats was evaluated (Pepelko et al., 1982b). Groups of 15 Sprague-Dawley rats were exposed to diluted (14:1) irradiated exhaust, to nonirradiated exhaust, or to clean air. Exposures were 20 h/d for 28 d. The exhaust was irradiated in a chamber illuminated with artificial sunlight to generate photochemical reactants. Concentrations of the exhaust components in the exposure chambers are given in Table 4-1. Body weight gains were significantly ($p < 0.05$) reduced in the exposed animals. Those animals exposed to irradiated exhaust showed a greater weight reduction than those exposed to nonirradiated exhaust. Arterial blood gases and standard bicarbonate were unaffected, but arterial blood pH was significantly ($p < 0.05$) decreased in animals exposed to irradiated exhaust. Residual volume

and wet lung weight were not altered by either exposure, but vital capacity and total lung capacity were increased significantly ($p < 0.05$) following exposure to the nonirradiated exhaust.

In related studies, Wiester et al. (1980) evaluated pulmonary function in 4-d old male and female Hartley guinea pigs exposed for 28 d to irradiated diesel exhaust (IE). The test animals (18 or 19 per test group) were exposed for 20 h/d, 7 d/week to the exhaust after dilution with clean air. The concentration of the exhaust components in the test chambers are given in Table 4-1. In comparison to controls, animals exposed for 28 d to the irradiated exhaust exhibited a 35 percent increase in pulmonary airflow resistance and a significant decrease in heart rate ($p < 0.002$). Dynamic compliance, minute volume, respiratory rate, and tidal volume were not affected. Histopathological changes in the lungs were evaluated in test animals exposed for 56 days to irradiated exhaust, to diluted raw exhaust (RE), or to clean air. The exposure resulted in a significant increase in the ratio of lung to body weight in both exposed groups (0.82 percent increase for RE and 0.78 percent for IE compared with 0.68 percent for controls). Heart/body weight ratio was not altered in either group. In both groups of exposed animals, black-pigmented macrophages accumulated in the alveoli and black particles were also seen in focal areas of the tracheobronchial tree and in bronchial and carinal lymph nodes. Hypertrophy of goblet cells in the tracheobronchial tree and focal hyperplasia of alveolar lining cells were also observed.

GM-Sponsored Studies. In studies conducted on male Fischer 344 rats (16 exposed and 8 controls), White and Garg (1981) found that short-term exposures (20 h/d, 5.5 d/week for up to 9 weeks) to diesel exhaust diluted to a particle concentration of 6.0 mg/m^3 resulted in an increase in soot-laden macrophages in the lungs after 6 h, an increase in Type II pneumocytes after 24 h, and an accumulation of inflammatory cells within the septa after 4 weeks. After 9 weeks of exposure, focal aggregations of soot-laden macrophages developed near terminal bronchi, along with an influx of polymorphonuclear leukocytes, Type II cell proliferation, and thickening of the alveolar walls. The affected alveoli occurred in clusters which for the most part were located near the terminal airways, but occasionally were focally located in the lung parenchyma. The exhaust was generated with a 5.7-L, four-

cycle, indirect injection engine operated at a steady speed and load (1350 rpm, 96 N•m, simulating 65 km/h) (Schreck et al., 1980).

Kaplan et al. (1982) evaluated the effects of subchronic exposure to diesel exhaust on Fischer 344 male rats, Syrian golden hamsters, and strain A/J mice. The exhaust was diluted to a particle concentration of 1500 $\mu g/m^3$, and the exposures were 20 h/d and

TABLE 4-1. COMPOSITION OF THE EXPOSURE ATMOSPHERES IN THE EPA STUDIES[a]

Exposure	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	Sulfate (mg/m^3)	THC[b] (ppm)	Ozone (ppm)
Control	0.00	2.0	0.07	0.11	0.0	0.0	0.0	0.0
Nonirradiated	6.32	17.4	2.3	5.9	2.1	0.57	31.6	0.0
Irradiated	6.83	16.7	2.9	5.0	1.9	0.57	26.1	<0.01

[a]Mean values.
[b]Total hydrocarbons.

Source: Wiester et al., 1980.

7 d/week. The hamsters were exposed for 86 d and the rats and mice for 90 d. Exhaust was generated by a 5.7-L Oldsmobile engine operated continuously at the equivalent of 40 mph. The concentrations of the gaseous components of the exhaust were not reported. There were no significant differences in mortality or growth rates between exposed and control animals. Lung weight relative to body weight of 15 rats exposed for 13 weeks was significantly higher ($p \leq 0.05$) than that for a group of control rats. Histological examination of tissues of all three species indicated particle accumulation in the lungs and mediastinal lymph nodes. A small increase occurred in the thickness of the alveolar walls without major histopathological changes. After 6 mo recovery, considerable clearance of the diesel particles from the lungs occurred in all three species.

4.1.2.3. Chronic Exposures.

4.1.2.3.1. *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.

EPA-Sponsored Studies. As part of an EPA-sponsored series of studies, Vinegar et al. (1980, 1981a, 1981b) investigated the effects of 6-mo exposure to diesel exhaust on adult male Chinese hamsters. The test animals (8 or 9 per group) were exposed 8 h/d, 7 d/week, for 6 mo to one of two dilutions of exhaust. One dilution (18:1) resulted in a particle concentration of 6 mg/m^3 and the other (9:1) resulted in a particle concentration of 12 mg/m^3. Neither exposure regime affected body weight gain of the hamsters; however, lung weights and lung/body weight ratios of the exposed animals were significantly greater than the controls ($p < 0.01$). The exhaust was generated by a 3.24-L Nissan engine operated in the Federal Short Cycle. The concentrations of exhaust components in the exposure chambers over the 6-m period were not reported, but concentrations for 2.5-yr-long tests conducted with the same exposure facilities and with the same exhaust dilution factors were reported by Pepelko (1982a). These are given in Table 4-2.

NIOSH-Sponsored Studies. As part of a series of studies evaluating the combined effects of diesel exhaust and coal dust on laboratory animals. Lewis et al. (1986) exposed rats and monkeys to diesel exhaust alone and followed changes in body weight over a 2-yr period. The exhaust was generated by a 425-in^3 displacement, four-cycle, water-cooled, naturally aspirated diesel engine (Caterpillar Model 3304) which developed 100 bhp (brake horsepower) at 2200 rpm. The engine was operated in an 8-mode (load/haul/dump) duty cycle typically used in underground coal mining. Idling constitued 60 percent of the cycle. The exhaust was passed through a Wagner water scrubber and then diluted with air by a factor of 27:1 before entering the exposure chambers. The particle concentration, measured with Real Time Aerosol Monitors, was about 2 mg/m^3.

TABLE 4-2. COMPOSITION OF THE EXPOSURE ATMOSPHERE IN THE EPA STUDIES [a]

Time Period	Particles (mg/m^3)	CO (ppm)	NO^2 (ppm)	NO (ppm)	SO^2 (ppm)	Acrolein (ppm)	Form. (ppm)	T H C [b] (ppm)
Control[c]	0.00	2.2	0.03	0.05	0.03	0.00	0.00	2.82
Weeks 1 to 61	6.34	20.17	2.68	11.64	2.12	0.025	0.106	7.93
Weeks 61 to 124	11.70	33.30	4.37	19.39	5.03	0.034	0.251	11.02

[a]Mean values.
[b]Total hydrocarbons.
[c]Weeks 1 to 124.

Source: Pepelko, 1982a.

The size distribution of the particles was determined with a Thermal-Systems Model 3030 electrical aerosol size analyzer. The mass median aerodynamic diameter of the particles was 0.23 and 0.36 μm by the instrumental and scanning electron microscope techniques, respectively. The geometric standard deviations were 2.5 and 2.0, respectively. Exposures were 7 h/d, 5 d/week. The test animals were either male and female Fischer 344 rats (72 or 144 per test) or male cynomolgus monkeys (15 per test). The concentrations of gaseous components of the exhaust were 11.5 ppm CO; 8.7 ppm NO; 1.5 ppm NO_2; 0.81 ppm SO_2; 60.2 ppb acrolein; 38.3 ppb formaldehyde; 29.0 $\mu g/m^3$ sulfates; and 7.5 ppm total hydrocarbons. After 24 mo of exposure, there were no significant changes in weight gain for either rats or monkeys.

DOE-Sponsored Studies. Karagianes et al. (1981) reported that Wistar rats (40 per exposure group and 40 controls) exposed for 20 mo to diluted diesel exhaust (particle concentration 8.3±2.0 mg/m^3, 0.71 μm MMAD (mass median aerodynamic diameter), 2.1 GSD) exhibited no significant changes in body weight or mortality pattern when compared with controls. Similar results were obtained with animals exposed to coal dust and diesel exhaust or to coal dust (14.9±6.2 mg/m^3). The test animals were exposed for 6 h/d and 5

d/week. The exhaust was generated from a 43-hp diesel engine operated under conditions simulating the use of underground mining vehicles. The concentration of CO was maintained at 50 ppm by using a 35:1 dilution with air. The NO_2 concentration ranged from 4 to 6 ppm, and the SO_2 and aliphatic aldehyde levels were below detection limits (1 ppm).

Lovelace Studies. Studies conducted at the Lovelace Inhalation Toxicology Research Institute on the chronic exposure of rats to diesel exhaust were reported on by McClellan et al. (1986). The exhaust was generated by a 5.7-L engine operated under the FTP urban driving cycle. Male and female F344 rats (initially 288 per test group) were exposed to three dilutions of diesel exhaust for 7 h/d, 5 d/week for 30 mo. The three exhaust dilutions were designed to achieve nominal particle concentrations of 0.35, 3.5, and 7 mg/m^3 (MMAD 0.23 to 0.26 μm). The concentrations of the other exhaust components in the test chambers are given in Table 4-3. The exposures had no apparent adverse effects on body weight or median life span of the rats.

GM-Sponsored Studies. In chronic exposure studies conducted at the General Motors Research Laboratories (GMR), the general health and body weight gain of male Fischer 344 rats and Hartley guinea pigs was not affected by 2-yr exposures to diesel exhaust diluted to achieve particle concentrations of 0.258, 0.796, or 1.533 mg/m^3 (0.1 to 0.3 μm MMAD) (Schreck et al., 1981). The exposures were for 20 h/day and 5.5 d/week. The exhaust was generated with a 5.7-L, four-cycle, indirect injection engine operated at a steady speed and load (1350 rpm, 96 N•m, simulating 65 kpm). Concentrations of the gaseous components of the exhaust were 3.4, 5.3, and 7.9 μg/m^3 CO, and 2.1, 5.0, and 9.2 μg/m^3 NO_x, for dilution levels of 240:1, 80:1, and 40:1, respectively. In related studies in which rats were exposed for up to 36 weeks to 0.25 or 1.5 mg/m^3, lung wet weight, normalized to body weight, was significantly higher than control values ($p < 0.01$) after 12 weeks of exposure (Misiorowski et al., 1980).

Fraunhofer Institut Studies. Heinrich et al. (1986a, 1986b; see also Stoeber, 1986) investigated the effects of long-term exposure to diesel exhaust on male and female Syrian golden hamsters, female SPF Wistar rats, and female NMRI mice. The exhaust was generated by a 1.6-L engine operated continuously according to the U.S. 72 (FTP)

TABLE 4-3. COMPOSITION OF THE EXPOSURE ATMOSPHERES IN THE LOVELACE STUDIES[a]

Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	Ammonia (ppm)	Hydrocarbons (ppm)
7.08	30	0.7	10	0.7	13
3.47	17	0.3	6	0.9	9
0.35	3	0.1	0.7	1.4	4
0.013[b]	1	0	0	1	3

[a]Mean of weekly values for 24 mo of exposure.
[b]Control.

Source: Henderson et al., 1988.

test driving cycle. The test fuel had a 0.36 percent sulfur content. The test animals (96 per exposure group and 96 per control group) were exposed for 19 h/d, 5 d/week. The maximum exposure period was 120 weeks for hamsters and mice, and 140 weeks for rats. After a 14:1 dilution with filtered air, the exhaust contained 4.24 mg/m^3 of particulate matter (0.3 μm MMAD), 2.5 ppm CO, 1.12 ppm SO_2, 1.5 ppm NO_2, and 11.4 ppm NO_x. Body weights of hamsters were not affected by exposure to diesel exhaust. Body weights of exposed rats and mice were reduced about 25 percent compared to controls, particularly in the second year of exposure. After 2 yr of exposure, higher mortality rates occurred in mice, and the wet and dry weights of the lungs of the exposed mice and rats were 2 to 3 times greater than the controls ($p < 0.05$). Lung weights of the hamsters increased 70 percent for the same time period.

CCMC Studies. The Committee of Common Market Automobile Constructors (CCMC) sponsored long-term rodent inhalation studies comparing the effects of diesel exhaust with those of gasoline exhaust. As part of this study, Fischer 344 rats were exposed for 2 yr (five 16-h periods per week) to diesel exhaust diluted to achieve particle

concentrations of 0.7, 2.2, and 6.6 mg/m^3 (Brightwell et al., 1986). The exhaust was generated with a 1.5-L engine operated under the U.S. 72 (FTP) test driving cycle. At the highest exposure the NO_x concentration was 8±1 ppm, and the CO concentration was 32±11 ppm (concentrations of other gaseous pollutants and for the other exposure levels were not given). At the two higher exposures, significant reductions were seen in the body weights of the exposed animals when compared with control animals. A significant, exposure-related increase in lung weight also occurred. Lung weights in males were 2.7 times the control values; those of females were 3.9 times the control values.

Ishinishi Studies. Ishinishi et al. (1986b) exposed male and female Fischer 344 rats to diesel exhaust from light-duty (LD) and heavy-duty (HD) diesel engines. The test animals (64, 95, or 120 per test group) were exposed for 16 h/d, 6 d/week, for 30 mo. The LD exhaust was generated from 1.8-L, 4-cylinder (swirl chamber) engines operated at 1200 rpm. The HD exhaust was generated from 11-L direct injection engines operated at 1700 rpm. The exhaust was diluted to achieve nominal particle concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 mg/m^3 (HD only). (Concentrations of other components of the diluted exhaust are given in Table 4-4). An exposure-related decrease in body weight was seen in both series. The survival rates in all exposed groups decreased between 24 and 30 mo.

4.1.2.3.2. *Effects on pulmonary function*

The effect of long-term exposure to diesel exhaust on pulmonary function has been evaluated in laboratory studies on hamsters, rats, guinea pigs, cats, and monkeys.

EPA-Sponsored Studies. Pepelko et al. (1980b, 1981; see also Pepelko, 1982a) and Moorman et al. (1985) measured lung function in adult male cats chronically exposed to diesel exhaust. The exhaust was generated with a four-cycle, 3.24-L, naturally aspirated Nissan engine operated in the 9-mode Federal Short Cycle and with No. 2 diesel fuel containing 0.15 percent sulfur (Hinners et al., 1980). During the first year of the tests, the exhaust dilution ratio was 18:1 and the resulting particle concentration was 6.34 mg/m^3 (by mass, 90 percent <1 μm, 50 percent <0.3 μm). The dilution ratio was decreased to 9:1 during the second year resulting in a particle concentration of 11.70 mg/m^3.

TABLE 4-4. COMPOSITION OF THE EXPOSURE ATMOSPHERES IN THE ISHINISHI STUDY

Diesel type	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	HTHC (ppm)	LTHC (ppm)	Formal (ppm)	SO_4 ($\mu g/m^3$)
LD[a]	0.003	0.80	0.01	0.03	0.06	2.20	2.17	0.002	0.41
	0.11	1.23	0.08	1.16	0.38	2.44	2.27	0.01	18.8
	0.41	2.12	0.26	3.81	1.06	2.93	2.51	0.03	62.4
	1.08	3.96	0.70	9.44	2.42	3.82	2.87	0.07	151
	2.31	7.10	1.41	18.93	4.70	5.49	3.57	0.13	315
HD[b]	0.002	0.63	0.02	0.04	0.06	2.43	3.50	0.003	0.49
	0.46	2.65	0.46	5.71	0.98	4.63	4.27	0.05	62.9
	0.96	4.85	1.02	12.11	1.79	7.15	5.16	0.11	111
	1.84	7.75	1.68	19.99	2.82	9.94	5.90	0.18	198
	3.72	12.91	3.00	34.45	4.57	15.65	7.62	0.29	361

[a]Light-duty diesel engine.
[b]Heavy-duty diesel engine.

Source: Ishinishi et al., 1986b.

Concentrations of other components of the diluted exhausts are given in Table 4-2. The test animals (21 per test group) were exposed to the exhaust 8 h/d and 7 d/week for up to 2.25 yr. Results were generally negative after the first year of exposure. After the second year, vital capacity was 369±42 mL in the exposed group and 410±58 mL in the control group, total lung capacity was 428±56 mL in the exposed group and 484±68 mL in the control group, and diffusing capacity was 0.90±0.27 mL/min/mm Hg in the exposed group and 1.01±0.14 mL/min/mm Hg in the control group. These differences were statistically significant at $p < 0.05$. Residual volume, maximum expiratory flow, compliance, resistance, and closing volume were not significantly different.

Vinegar et al. (1980, 1981a, 1981b) investigated the effects of diesel exhaust on lung function in adult male Chinese hamsters. The test animals (8 or 9 per group) were exposed 8 h/d, 7 d/week, for 6 mo to either of two dilutions of exhaust. One dilution (18:1) resulted in a particle concentration of 6.4-mg/m^3 and the other (9:1) resulted in a particle concentration of about 12 mg/m^3. The exhaust was generated by a 3.24-L Nissan engine operated under the Federal Short Cycle. The concentrations of exhaust components in the exposure chambers over the 6-mo period were not reported, but concentrations for 2.5-yr-long tests conducted with the same exposure facilities and with the same exhaust dilution factors were reported by Pepelko (1982a). These are given in Table 4-2. Vital capacity, vital capacity/lung weight ratio, residual lung volume by water displacement, and carbon dioxide diffusing capacity decreased significantly ($p < 0.01$) in the animals exposed to the 6.4 mg/m^3 level of particulate matter. Static deflation lung volume, expressed as percent vital capacity, was increased at 0 and 5 cm H_2O. Similar effects were reported to occur at the 9:1 dilution (Vinegar et al., 1981a), but detailed information was not provided.

NIOSH-Sponsored Studies. As part of a NIOSH-sponsored study evaluating the combined effects of diesel exhaust and coal dust on laboratory animals, Green et al. (1983) and Lewis et al. (1986) measured changes in pulmonary function in rats and monkeys exposed to diluted diesel exhaust in the absence of coal dust. The exhaust was generated by a 7.0-L, four-cycle Caterpillar engine using fuel with <0.5 percent sulfur. The engine was equipped with a water scrubber and operated in a repetitive 8-mode coal mining duty cycle. About 55 percent of the cycle was in the idle mode. Exposures were for 7 h/d, 5 d/week for up to 2 yr. Male and female Fischer 344 rats (72 or 144 per test group) and male cynomolgus monkeys (72 per test group) were used in the tests. The exhaust was diluted with air (27:1) to achieve a particle concentration of 2 mg/m^3 (0.23 and 0.36 μm MMAD by the instrumental and scanning electron microscope techniques; the geometric standard deviations were 2.5 and 2.0, respectively). The concentrations of other exhaust pollutants in the test chamber are given in Table 4-5. Results of the physiological tests conducted on the rats (10 per test group) exposed to diesel exhaust alone revealed no evidence of impairment of pulmonary function after 12 mo (Green et al., 1983). After 24 mo of exposure to the diesel exhaust in the absence of coal dust, there were no significant

TABLE 4-5. COMPOSITION OF THE EXPOSURE ATMOSPHERE IN THE LEWIS STUDY

Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	Sulfates ($\mu g/m^3$)	Ammonia (ppm)	Acrolein (ppm)	Formal. (ppm)	THC (ppm)
2.01[a]	12.7	1.6	9.7	0.83	—	1.13	—	—	—
control	2.3	0.04	0.07	0.01	—	0.63	—	—	—
1.95[b]	11.5	1.5	8.7	0.81	29.0	0.64	0.06	0.038	7.5
control	2.2	0.06	0.08	—	—	—	0.003	0.008	4.1

[a]Average values for 12 mo (Green et al., 1983).
[b]Average values for 24 mo (Lewis et al., 1986).

changes in residual capacity, airway resistance, and alveolar morphometry (Lewis et al., 1986). In tests on 15 monkeys, forced expiratory flow at 50 percent of vital capacity/forced vital capacity was significantly lower in animals exposed to diesel exhaust alone for 18 mo ($p = 0.02$) compared with controls. Forced expiratory flow at 40 percent of total lung capacity/total lung capacity was significantly lower in animals exposed for 24 mo ($p = 0.05$).

Lovelace Studies. Diesel exhaust studies conducted at Lovelace Inhalation Toxicology Research Institute included an evaluation of the effects of chronic inhalation exposures on pulmonary function of male and female Fischer 344 rats (Mauderly et al., 1985a, 1985b; see also McClellan et al., 1986). In these studies the exhaust was generated by a 5.7-L engine operated under the FTP urban driving cycle. Three exhaust dilutions were used which were designed to achieve nominal particle concentrations of 0.35, 3.5, and 7 mg/m^3 (MMAD 0.23 to 0.26 μm). The concentrations of other exhaust components in the test chambers are given in Table 4-3. Exposures were for 7 h/d, 5 d/week, for up to 30 mo. 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 (DLC), FVC, and CO diffusing capacity than controls ($n = 23$). After 30 months of exposure to 7 mg/m^3, mean TLC, DLC, quasistatic chord compliance, and CO diffusing capacity were significantly lower than control values. Nitrogen washout and percent of FVC expired in 0.1 s were

significantly greater than control values (p <0.05). There was no evidence of airflow obstruction. The functional alterations were attributed to focal fibrotic, proliferative lesions, and thickened alveolar membranes which were observed by histological examination.

GM-Sponsored Studies. The effect of diesel exhaust on the pulmonary function of 25 male Fischer 344 rats was investigated in a series of chronic toxicity tests conducted at the General Motors Research Laboratories. The exposure system, engine operating conditions, and characterization of the exposure atmospheres were described by Schreck et al. (1980, 1981). The exhaust was generated with a 5.7-L, four-cycle, indirect injection engine operated at a steady speed and load (1350 rpm, 96 N•m) simulating 65 km/h. The pipes in the exhaust delivery system were heated to 100±15°C to simulate typical tailpipe conditions. The exhaust was then diluted with clean air to achieve a particle concentration of 1.5 mg/m^3 (0.2 μm MMAD). The diluted exhaust also contained 7.9 μg/m^3 (7 ppm) CO and 9.2 μg/m^3 NO_x. Exposures were for 20 h/d and 5.5 d/week for seven exposure periods up to 612 d. No significant alterations in pulmonary function occurred after 1 y (Gross, 1981a); however, after 612 d of exposure, 6 of the 18 measured parameters showed statistically significant elevations in the exposed animals when compared with controls. Functional residual capacity and its component volumes, expiratory reserve and residual volume, maximum expiratory flow (MEF) at 40 percent FVC, MEF at 20 percent FVC and $FEV_{0.1}$, were significantly greater in the exposed animals (Gross, 1981b).

Fraunhofer Institut Studies. Heinrich et al. (1982) determined the effects of diesel exhaust on the pulmonary function of female Wistar rats. The exhaust was generated with a 2.4-L Daimler-Benz engine operated at a constant load of 16 kW and a uniform speed of 2400 rpm. The diesel fuel contained 0.36 percent sulfur. The exhaust was diluted to achieve a particle concentration of 3.9 mg/m^3 (0.1 μm MMAD). The concentrations of the other components of the diluted exhaust, as well as the pollutant levels in test chambers using filtered exhaust (discussed in Section 5.2.1) are given in Table 4.6. The test animals (number per group not given) were exposed for 2 years. The exposure period was 7 to 8 h/d, 5 d/week. When compared with a control group, no significant changes in respiratory rate, respiratory minute volume, or compliance and resistance occurred in the exposed group.

Additional studies were conducted by Heinrich et al. (1986, see also Stoeber, 1986); on the effects of long-term exposure to diesel exhaust on pulmonary function of male and female Syrian golden hamsters and female SPF Wistar rats. The exhaust was generated by a 40-kW, 1.6-L engine operated under the U.S. 72 (FTP) test driving cycle. The fuel contained 0.36 percent sulfur. The exhaust was diluted to achieve a particle concentration of 4.24 mg/m^3 (0.35 μm MMAD). The concentration of the other components of the diluted exhaust are given in Table 4.7. The animals (96 per test and control groups) were exposed for 19 h/d, 5 d/week, for a maximum of 120 weeks (hamsters) or 140 weeks (rats). After 1 yr of exposure to the unfiltered diesel exhaust, the hamsters exhibited 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. These parameters did not show any further changes after 2 yr of exposure. After 2 yr, dynamic lung compliance was 0.190±0.02 mL/cm H_2O in the exposed group and 0.281±0.09 mL/cm H_2O in the controls (p <0.05), and airway resistance was 0.628±0.10 cm H_2O/mL/s in the exposed group and 0.544±0.10 cm H_2O/mL/s in the controls (p <0.05).

CCMC Studies. Brightwell et al. (1986) evaluated the effect of chronic inhalation of diesel exhaust on the pulmonary function of Syrian hamsters and Fischer 344 rats. The test animals (54 male and 54 female hamsters and 72 male and 72 female rats per test group) were exposed for five 16-h periods per week for 2 yr. The exhaust was generated with a 1.5-L engine operated under the U.S. 72 (FTP) test driving cycle. Three exhaust dilutions were tested, producing particle concentrations of 0.7, 2.2, and 6.6 mg/m^3. At the highest exposure the NO_x concentration was 8±1 ppm, and the CO concentration was 32±11 ppm (concentration of other gaseous pollutants and for the other exhaust dilutions was not reported). There were no treatment-related changes in pulmonary function in the hamsters. Rats exposed to the highest concentration of exhaust exhibited changes in respiratory physiology (data not reported) which were reported to be indicative of restrictive and obstructive airway disease.

TABLE 4-6. COMPOSITION OF THE EXPOSURE ATMOSPHERE IN THE HEINRICH 1982 STUDY[a]

	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	THC[b] (ppm)
Unfiltered	3.9	18.5	1.2	16.5	3.1	9.3
Filtered	—	18.0	1.0	17.2	2.8	7.9

[a]Mean values.
[b]Total hydrocarbons.

Source: Heinrich et al., 1982.

TABLE 4-7. COMPOSITION OF THE EXPOSURE ATMOSPHERE IN THE HEINRICH 1986 STUDY[a]

	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	THC[b] (ppm)
Unfiltered	4.24	12.5	1.5	10.0	1.12	5.5
Filtered	—	11.1	1.2	8.7	1.02	5.2
Control	—	0.16	—	—	—	3.5

[a]Mean values.
[b]Total hydrocarbons.

Source: Heinrich et al., 1986a.

4.1.2.3.3. *Lung morphology, biochemistry, and lung lavage analysis*

Several series of investigations have examined the morphological, histological, and histochemical changes occurring in the lungs of animals chronically exposed to diesel exhausts.

EPA-Sponsored Studies. EPA has conducted chronic diesel exhaust studies on several species of laboratory animals including hamster, cats, rats, and mice. In these studies the exhaust was generated with a four-cycle, 3.24-L, naturally aspirated Nissan engine operated in the 9-mode Federal Short Cycle, with No. 2 diesel fuel containing 0.15 percent sulfur. The test animals were exposed for 8 h/d or 20 h/d for 7 d/week, and the exhaust was diluted with clean air at ratios of 18:1 or 9:1 to achieve nominal particle concentrations of 6.0 or 12 mg/m^3 (by mass 90 percent <1 μm, 50 percent <0.3 μm). The composition of the exposure atmospheres is shown in Table 4-2.

In studies conducted on Chinese hamsters, Pepelko (1982a) found that animals exposed 8 h/d, 7 d/week, for six mo to particle levels of 6 or 12 mg/m^3 exhibited lungs characterized by large numbers of black alveolar macrophages in the alveolar spaces; thickening of the alveolar lining; hyperplasia of Type II alveolar cells; and edema.

In an EPA study conducted on cats, the exposure level (exhaust particle concentration) was increased from 6.34 mg/m^3 during the first year of the tests to 11.70 mg/m^3 during the second year (Plopper et al., 1983; Hyde et al., 1985). The 27-mo-long exposure resulted in an accumulation of exhaust particles in macrophages in the alveolar sacs and interstitial spaces. Type II cell hyperplasia was seen in the proximal interalveolar septa (Plopper et al., 1983). Biochemical analysis indicated increased synthesis of lung collagen. There was a 2.7-fold increase in the thickness of the peribronchial connective tissue space in the proximal acinar region. Significant (p <0.05) increases were seen in numbers of extracellular fibers, interstitial macrophages, and lymphocytes. The peribronchiolar fibrosis increased in severity even after 6 mo of recovery in clean air (Hyde et al., 1985).

In related EPA-sponsored studies utilizing the same exposure system as that described above, Bhatnagar et al. (1980; see also Pepelko, 1982a) evaluated changes in the biochemistry of lung connective tissue of exhaust-exposed male Sprague-Dawley CD rats and

A/HEJ mice. The mice were exposed for 8 h/d and 7 d/week, and the rats for 20 h/d and 7 d/week for up to 9 mo, to exhaust containing 6.34 mg/m^3 particulate matter. Total lung protein in rats exposed for 42 d increased about 40 percent over that of controls. *In vivo* leucine incorporation was decreased, suggesting a decrease in overall protein synthesis. *In vivo* proline incorporation, an estimate of collagen synthesis, was not affected by the exposure. Prolyl-hydroxylase activity was increased in rats exposed 42 d and in rats exposed *in utero*, suggesting increased collagen synthesis. In mice exposed to diesel exhaust for up to 9 mo, large increases in lung protein content and collagen synthesis were found, but overall protein synthesis decreased. The increase in collagen synthesis suggested proliferation of connective tissue and possible fibrosis (Pepelko, 1982a).

NIOSH-Sponsored Studies. The chronic effects of diesel exhaust on laboratory animals was investigated as as part of a NIOSH-sponsored study evaluating the combined effects of diesel exhaust and exposure to coal dust (Green et al., 1983; Castranova et al., 1985; Fedan et al., 1985; and Lewis et al., 1986). Exposures were for 7 h/d, 5 d/week, for up to 2 yr. The test animals were either male and female Fischer 344 rats (72 or 144 per test) or male *cynomolgus* monkeys (15 per test). The diesel exhaust was generated by a four-cycle, Caterpillar engine equipped with a water scrubber and operated in a repetitive 8-mode coal mining duty cycle. The concentration of particulate matter was about 2 mg/m^3 (0.23 and 0.36 μm MMAD by the instrumental and scanning electron microscope techniques, with corresponding GSDs of 2.5 and 2.0, respectively). The concentrations of other exhaust components, as reported in the separate studies were: 10.5 to 11.5 ppm CO; 7.8 to 9.7 ppm NO; 1.5 to 1.6 ppm NO_2; 0.6 to 0.83 ppm SO_2; 60.2 ppb acrolein; and 38.3 ppb formaldehyde. Histological examination of rat lung tissue showed that particles accumulated in macrophages in the alveolar sacs after 3 mo of exposure to the diesel exhaust in the absence of coal dust. Accumulations increased up to 12 mo, at which time the particles were also being incorporated into the walls of the respiratory bronchioles. No changes were seen in cytochrome P450 levels in the lung microsomes of exposed rats. The physiological viability of alveolar macrophages of exhaust-exposed rats was assessed after 2 yr of exposure by Castranova et al. (1985). The exposure had little effect on the following: viability, cell

number, oxygen consumption, membrane integrity, lysosomal enzyme activity, and protein content of the macrophages. A slight decrease in cell volume, a decrease in chemiluminescence, and a decrease in ruffling of the cell membrane was observed. The investigators attributed the latter effects to an overall reduction in macrophage activity. Histopathological studies of rats exposed for 24 mo revealed multifocal histiocytosis (pulmonary lipoidosis) occurring primarily subpleurally and composed of collections of degenerating foamy macrophages, as well as amorphous granular material together with fibrosis and chronic inflammatory cells. These lesions involved both the interstitium and alveolar spaces. Hyperplasia of alveolar epithelial Type II cells and degeneration of Type I cells was also observed. In the exposed monkeys, there was perivascular, peribronchiolar and alveolar accumulation of particles, but no evidence of fibrosis, focal emphysema, or inflammation. Exposure of rats and monkeys to coal dust alone (4.98±0.82 mg/m^3, total particles, and 2.00±0.41 mg/m^3 respirable particles) resulted in histopathological changes similar to those seen in the diesel exhaust assays; however, there was less evidence of lipoidosis. Coal dust increased the number of alveolar macrophages obtained by lavage, enhanced the secretion of reactive forms of oxygen, and increased cellular surface area. In contrast, diesel exhaust caused a reduction in the secretion of reactive forms of oxygen and diminished surface ruffling. In animals exposed to both diesel exhaust and coal dust there was no evidence of synergistic effects.

DOE-Sponsored Studies. As part of another study examining the combined effects of diesel exhaust and coal dust, Karagianes et al. (1981) investigated the effects of inhaled diesel exhaust (in the absence of coal dust) on the lungs of 18-week-old, male Wistar rats. The test animals (6 per test group) were exposed for 6 h/d, 5 d/week, for 4, 8, 16 or 20 mo. The exhaust was diluted to achieve a particle concentration of 8.3±2.0 mg/m^3 (0.71 μm MMAD, 2.1 GSD). Histological examination of lung tissue indicated focal aggregation of soot-laden alveolar macrophages, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema (methodology not described). Lesion severity was related to length of exposure. Statistical analysis of lesion severity after 20 mo indicated no significant difference (p = 0.05) between diesel exhaust, diesel exhaust plus coal dust (5.8±3.5 mg/m^3), or high concentration (14.9±6.2 mg/m^3) coal dust exposure groups. The exhaust was generated from a 43-bhp

diesel engine operated under conditions simulating the use of underground mining vehicles. The concentration of CO was maintained at 50 ppm by using a 35:1 dilution with air. The concentration of NO_2 was 4 to 6 ppm, and the levels of SO_2 and aliphatic aldehydes were below the detection limit of 1 ppm.

Lovelace Studies. Diesel exhaust studies conducted on rats and mice at the Lovelace Inhalation Toxicology Research Institute included analyses of biochemical and cytological changes in lung tissue and bronchoalveolar lavage fluid (BALF). Results of these studies appeared in several reports (Henderson et al., 1983; McClellan et al., 1986; Mauderly et al. 1987b; Henderson et al., 1988). The exhaust was generated by a 5.7-L engine operated under the FTP urban driving cycle. Three exhaust dilutions were selected to achieve nominal particle concentrations of 0.35, 3.5, and 7 mg/m^3 (0.23 to 0.26 μm MMAD). The concentrations of other exhaust components in the test chambers are given in Table 4-3. The test animals were male and female Fischer 344 rats (initially 288 per test group) and CD-1 mice (initially 360 per test group). Exposures were for 7 h/d, and 5 d/week. The test period was 2 yr in the rat study and 18 mo in the mouse study. At the lowest exposure level, no biochemical or cytological changes occurred in BALF or in lung tissue in either species. 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 ß-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 1 yr of exposure at 7 mg/m^3, the lungs of the rats exhibited focal areas of fibrosis, whereas the lungs of the mice exhibited only a fine fibrillar thickening of a few alveolar septa. Foci of epithelial metaplasia occurred uniformly in the alveoli and terminal bronchioles of rats exposed to 7.0 mg/m^3 and were more scattered in the lungs of rats exposed to 3.5 mg/m^3. Rats exposed for 24 mo to exhaust containing a particle concentration of 3.5 mg/m^3 had a five-fold increase in the bronchoconstrictive prostaglandin PGF2a and twofold increases in the inflammatory leukotriene LTB4 (Henderson et al., 1985). In similarly exposed mice, there was a twofold increase in both parameters. The investigators concluded that the release of

larger amounts of these mediators of inflammation from the alveolar phagocytic cells of the rats accounted for the greater fibrogenic response seen in that species.

In a continuation of the diesel exhaust studies carried out at the Lovelace Inhalation Toxicology Research Institute, Mauderly et al. (1987a) evaluated the relative susceptiblity of developing lungs to damage by diesel exhaust and nitrogen dioxide. Male Fischer 344 rats (48 per test group) were exposed to either diesel exhaust or NO_2 (9.5 ppm). Exposures were 7 h/d and 5 d/week through gestation to the age of 6 mo, or from the age of 6 to 12 mo. The exposure atmospheres are given in Table 4-8. Comparative studies were conducted on respiratory function, immune response, lung clearance, airway fluid enzymes, protein and cytology, lung tissue collagen and proteinase, lung burdens of diesel soot, and lung morphology and histopathology. Exposure to diesel exhaust altered airway fluid enzyme levels, as well as lung collagen and proteinases in both age groups. After the 6-mo exposure, adult rats, when compared with controls, exhibited: (1) focal aggregates of soot-containing macrophages in the alveolar ducts near the terminal bronchioles; (2) a sixfold increase in the neutrophils (as percentage of total leukocytes) in the airway fluids; (3) a significantly ($p < 0.05$) higher number of total lymphoid cells in the pulmonary lymph nodes; (4) delayed clearance of diesel soot and radiolabelled particles ($t_{½}$ = 90 d vs. 47 d for controls); and (5) increased lung weights. These effects were not seen in the young rats. On a weight for weight basis, diesel soot accumulation in the lungs was similar in young and adults immediately after the exposure. During a 6-mo postexposure period, soot clearance was much more rapid in the young rats. During this time, soot-laden macrophages became aggregated in the young rats but these aggregations were located primarily in a subpleural position. Mauderly et al. (1987a) concluded that, in terms of pulmonary function, structure, and biochemistry, developing rats were not affected more severely than the adult rats by the diesel exhaust exposures.

GM Studies. Studies on the effects of chronic inhalation of diesel exhaust on male Fischer 344 rats and male Hartley guinea pigs were conducted at the General Motors Research Laboratories. The exposures were 20 h/d, 5.5 d/week, for up to 2 yr. The exhaust was generated with a 5.7-L, four-cycle, indirect injection engine operated at a steady speed and load (1350 rpm, 96 N • m), simulating 40 mph (Schreck et al., 1981). The test fuel had

TABLE 4-8. COMPOSITION OF EXPOSURE ATMOSPHERES IN THE MAUDERLY et al., 1987a STUDY[a]

	Particles (mg/m^3)	NO_x (ppm)	NO_2 (ppm)	CO (ppm)	HC[b] (ppm)
Exhaust study:					
develop.	3.5	5.2	—	7.9	4.6
adults	3.55	5.0	—	7.9	4.7
NO_2 study:					
develop.	—	—	9.5	—	—
adult	—	—	9.5	—	—
Control	0.006	—	—	—	—

[a]Mean values.
[b]Hydrocarbon vapors.

Source: Mauderly et al., 1987a.

a sulfur content of 0.27 percent. The pipes in the exhaust delivery system were heated to 100±15°C to simulate typical tailpipe conditions. The exhaust was then diluted with clean air (dilution ratios 240:1, 80:1, and 40:1) to achieve nominal particle concentrations of 0.25, 0.75, and 1.5 mg/m^3. The mean concentrations for the entire 2-yr period were 0.258. 0.796, and 1.533 mg/m^3 (0.2 μm MMAD, with 90 percent of the mass associated with particles less than 1.0 μm). The exposure chambers also contained 3.4 $\mu g/m^3$ CO and 2.1 $\mu g/m^3$ NO_x at 0.258 mg/m^3, 5.3 $\mu g/m^3$ CO and 5.0 $\mu g/m^3$ NO_x at 0.796 mg/m^3, and 7.9 $\mu g/m^3$ CO and 9.2 $\mu g/m^3$ NO_x at 1.533 mg/m^3.

Histological changes in the lungs of the guinea pigs used in the GM tests were reported on by Barnhart et al., 1981; 1982). In a preliminary study exposures to 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo resulted in uptake of exhaust particulate matter by three alveolar cell types (alveolar macrophages, epithelial Type I cells, and interstitial macrophages) and also by granulocytic leukocytes (eosinophils). The alveolar-capillary boundary increased in thickness as a result of an increase in absolute tissue volume of interstitium and epithelial Type II cells. In a continuation of these studies guinea pigs were

exposed to diesel exhaust (including exhaust diluted to achieve a particle concentration of 6.0 mg/m^3) for 2 yr (Barnhart et al., 1982). A minimal tissue response occurred at the concentration of 0.25 mg/m^3 (i.e., after 9 mo, epithelial Type I and II cells, endothelial cells, and interstitial cells increased 30 percent over concurrent age-matched controls ($p < 0.05$); by 24 mo only macrophages and epithelial Type II cells were significantly increased). As in the earlier study, ultrastructural evaluation indicated that epithelial Type I cells and macrophages in the alveolar parenchyma and granulocytic leukocytes (eosinophils) phagocytized the exhaust particles. Exposure to 0.75 mg/m^3 for 6 mo resulted in histological evidence of fibrosis in areas of macrophage clustering and in focal epithelial Type II cell proliferation. With increasing exposure levels, epithelial Type II cell clusters occurred in some alveoli. Excessive secretory products were seen in the alveolar spaces and were particularly prominent after exposure to 1.5 and 6.0 mg/m^3.

As part of the GM studies, Wallace et al. (1987) determined the cellular makeup of the intravascular and interstitial spaces of the lungs of exposed guinea pigs and rats. The test animals (three per group) were exposed for 2, 6, 10, or 72 weeks. After 18 mo, significant increases occurred in alveolar macrophages and in mononuclear interstitial cells at all exposure levels. From the 6th week through the 18th mo of exposure, both species had slight increases in fibroblasts in the interstitial areas.

Another part of the GM studies involved the evaluation of the exhaust effects of the exhaust on the types and numbers of cells lavaged from the lungs of the exposed animals. In one series of tests, rats and guinea pigs were exposed to exhaust particle levels of 0.25, 1.5, or 6.0 mg/m^3 for up to 12 mo (Chen et al., 1980). The exposures resulted in increases in the size and relative surface area of lavaged alveolar macrophages, and in an apparent decrease in phagocytizing ability. Exudative leukocytes (eosinophils in guinea pigs and neutrophils in rats) appeared in the lavage fluid in numbers which increased with exposure level and duration. Lavage fluid from exposed guinea pigs also contained increased numbers of "reactive" monocytes, macrophages characterized by atypically lobulated or multiple nucleii. In related studies, rats were exposed for 26 and 48 weeks to 0.25 and 1.5 mg/m^3, or for 24.5, 28, and 52 weeks to 0.75 mg/m^3 (Vostal et al., 1981, 1982; Strom, 1984). Exposure-related changes occurred in alveolar macrophages, polymorphonuclear leukocytes

(PMN), and lymphocytes in the lung lavage fluid. At a particle concentration of 0.25 mg/m^3, cell counts of alveolar macrophages were not different from control values, at 0.75 mg/m^3, the counts were 50 percent higher than the controls; and at 1.5 mg/m^3, counts were twice as high as the controls. The data suggested that the response of the alveolar macrophages was concentration dependent. Polymorphonuclear leukocytes were also present in the lung lavage fluid of animals exposed to 0.75 and 1.5 mg/m^3. Mononuclear leukocytes (which appeared to be lymphocytes) were found in the fluid after 1 yr of exposure. Protein, ß-glucuronidase activity, and acid phosphatase activity were significantly elevated in lavaged cells from animals exposed for 6 mo to 0.75 and 1.5 mg/m^3. In related tests on rats and guinea pigs, Weller et al. (1981) found that acid phosphatase activity in lavaged alveolar macrophages from exposed animals was reduced in an exposure-dependent (0.25 and 1.5 mg/m^3) and time-dependent (1 d, 2 weeks, and 12 mo) manner.

In the same series of GM tests, the lungs of animals exposed to the exhaust were also analyzed for biochemical changes (Misiorowski et al., 1980; Misiorowski et al., 1981; Eskelson et al., 1981). In most cases, exposures to 0.25 mg/m^3 did not cause any significant changes. DNA content in lung tissue and the rate of collagen synthesis were significantly increased at the highest exposure (1.5 mg/m^3) 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 only 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 animals exposed to 0.75 mg/m^3 and in lung cholesterol in animals exposed to 1.5 mg/m^3. Pulmonary prostaglandin dehydrogenase activity in guinea pigs was stimulated by an exposure for 6 weeks to 0.25 mg/m^3, but was not affected by exposure to 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. Schneider and Felt (1981) reported that similar exposures did not substantially change adenylate cyclase and guanylate cyclase activities in lung or liver tissue of the exposed rats and guinea pigs.

In related GM-sponsored studies, Chaudhari and Dutta (1982) investigated whether diesel exhaust-induced lung damage would result in the release of angiotensin converting

enzyme (ACE) from lung endothelial tissue as is the case for pulmonary edemagenic agents such as paraquat. Lung ACE activity decreased significantly after 8 weeks of exposure to 6.0 mg/m^3. ACE activity in serum increased significantly after 4 weeks of exposure and showed a much greater increase after 8 weeks. Chaudhari and Dutta (1982) suggested that the changes in ACE activity in the lung and serum were indicative of damage to pulmonary endothelial cells.

Fraunhofer Institut Studies. Chronic inhalation toxicity studies on diesel exhaust have been conducted at the Fraunhofer Institut für Toxikologie und Aerosolforschung. The results of these studies had been reported by Heinrich et al. (1982, 1985, 1986a, 1986b). In one set of tests, the exhaust was generated with a 2.4-L Daimler-Benz engine operated at a constant load of 16 kW and a uniform speed of 2400 rpm. The diesel fuel contained 0.36 percent sulfur. The concentrations of the other components of the diluted exhaust are given in Table 4-6. The test animals were female Syrian golden hamsters (48 per test group). Exposures were 7 to 8 h/d, 5 d/week, to diesel exhaust diluted to achieve a particle concentration of 3.9 mg/m^3 (0.1 μm MMAD). Histological examination of the respiratory tract revealed discrete proliferative changes in the lung (Heinrich et al., 1982, 1985). Sixty percent of the changes were described as adenomatous proliferations. Both control and test animals exhibited increasingly serious degrees of amyloidosis of the kidneys, adrenals, liver, and spleen. Cysts were also observed in the liver.

Heinrich et al. (1986a; see also Stoeber, 1986) conducted additional long-term inhalation studies on hamsters, mice, and rats. The exhaust was generated by a 40-kW, 1.6-L engine operated under the U.S. 72 (FTP) test driving cycle. The fuel contained 0.36 percent sulfur. The concentrations of the other components of the diluted exhaust are given in Table 4-7. The test animals consisted of 96 male and female Syrian golden hamsters, 96 female NMRI mice, and 96 female SPF Wistar rats per test group. Exposures were for 19 h/d, 5 d/week for up to 2 years. The exhaust was diluted to achieve a particle concentration of 4.24 mg/m^3 (0.35 μm MMAD). The maximum exposure period was 120 weeks for hamsters and mice and 140 weeks for rats. Histopathological examination indicated that different levels of response occurred in the three species. In hamsters, the exhaust caused thickened alveolar septa, bronchiolo-alveolar hyperplasia, and what were termed

emphysematous lesions. In mice, bronchiolo-alveolar hyperplasia occurred in 64 percent of the animals exposed to the exhaust and in 5 percent of the controls. Multifocal alveolar lipoproteinosis occurred in 71 percent and multifocal interstitial fibrosis occurred in 43 percent of the animals exposed to exhaust, but in only 4 percent or less of the controls. In the exposed rats there were severe inflammatory changes in the lungs, as well as thickened septa, foci of macrophages, and hyperplastic and metaplastic lesions. Biochemical studies of lung lavage fluids revealed that the exposures caused significant ($p \leq 0.05$) increases in lactate dehydrogenase, alkaline phosphatase, acid phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), total protein, protease (pH 5.1), and collagen in the hamsters and rats. Cytological studies revealed a significant increase in macrophages in lung lavage fluid of rats and hamsters after 1 yr of exposure, but not after 2 yr. Total leukocytes increased significantly in both species after 1 and 2 yr of exposure ($p \leq 0.05$). In hamsters, significant increases in granulocytes and lymphocytes in the lung lavage fluid were seen after 2 yr of exposure ($p \leq 0.05$).

Ishinishi Studies. Histopathological effects of diesel exhaust on the lungs of rats have been investigated by Ishinishi et al. (1986b) who compared the effects of both light-duty (LD) and heavy-duty (HD) diesel engines. The LDD exhaust was generated from 1.8-L 4-cylinder (swirl chamber) engines operated at 1200 rpm. The HDD exhaust was generated from 11-L direct injection engines operated at 1700 rpm. The exhaust was diluted to achieve nominal particle concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 mg/m^3 (HD only). Particle size was not reported. Concentrations of other components of the diluted exhausts are given in Table 4-4. The test animals (male and female F344 rats; 64, 95, or 120 per test group) were exposed for 16 h/d, 6 d/week, for 30 mo. No histopathological changes were observed in the lungs of rats exposed to particle concentrations less than 0.4 mg/m^3. At concentrations above 0.4 mg/m^3, severe morphological changes were seen. These changes consisted of marked hyperplasia of Type II epithelial cells and development of lung adenomas and carcinomas. Pathological lesions consisting of shortened and absent cilia in the tracheal and bronchial mucosal epithelium were also observed in the exposed animals. These lesions appeared to increase in severity with increase in exhaust concentration and period of exposure.

Iwai Studies. Histopathological effects of diesel exhaust on the lungs of rats have also been investigated by Iwai et al. (1986). The exhaust was generated from a 2.37-L diesel engine operated at 1000 rpm and 80 percent engine load. The exhaust was diluted (1:10) with clean air so that the exposure atmosphere contained 4.9±1.6 mg/m^3 of particulate matter, 30.9±10.9 ppm NO_x, 1.8±1.8 ppm NO_2, 13.1±3.6 ppm SO_2, and 7.0±1.4 ppm CO. The test animals were Fischer 344 female rats. The exposures were 8 h/d, 7 d/week for 24 mo. At 1 and 3 mo of exposure, there were only minimal histological changes in the lungs of the exposed animals. After 6 mo of exposure, there were noticeable numbers of particle-laden macrophages distributed irregularly throughout the lung and a proliferation of Type II alveolar epithelial cells with adeomatous metaplasia in areas where the macrophages had accumulated. After 1 yr of exposure, foci of heterotropic hyperplasia of ciliated or nonciliated bronchiolar epithelium on the adjacent alveolar walls were more common, the quantity of deposited particulate matter increased, and there was an increase in the number of degenerated alveolar macrophages and in the number of proliferative lesions of Type II alveolar or bronchiolar epithelial cells. After 2 yr of exposure, there was a fibrous thickening of the alveolar walls and mast cell infiltration with epithelial hyperplasia in areas where the macrophages had accumulated. Neoplastic changes occurred, resulting in two types of lung carcinoma, adenocarcinoma and squamous or adenosquamous carcinoma.

4.1.2.3.4. *Effects on the liver*

Meiss et al. (1981) investigated the effects of diesel exhaust on the liver of Syrian golden hamster exposed 7 to 8 h/d, 5 d/week for 5 mo to exhaust diluted 1:5 (or 1:10) with clean air. The diluted exhaust contained 11 (4) mg/m^3 of particulate matter, 25 (12) ppm CO, 43 (23) ppm NO, 1.5 (0.5) ppm NO_2, 7 (3) ppm SO_2, and 12 (8) ppm hydrocarbons. The livers of animals exposed to both dilutions exhibited moderate dilatation of the sinusoids with activation of the Kupffer cells and slight changes in nuclei. Fatty deposits were observed in the sinusoids, and small fat droplets were occasionally observed in the peripheral hepatocytes. Mitochrondria often showed a loss of cristae and exhibited 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.

4.1.2.3.5. *Effects on defense mechanisms*

A number of laboratory studies have demonstrated that exposure to diesel exhaust can alter resistance to respiratory tract infections.

EPA-Sponsored Studies. Campbell et al. (1980, 1981) recorded the mortality response of mice exposed to diesel exhaust following infectious challenge with *Salmonella typhimurium*, *Streptococcus pyogenes*, or A/PR8-3 influenza virus. The test animals (20 per group) were young adult female, CR/CD-1 albino mice. The exposure period was 8 h/d, 7 d/week, for up to 321 d. The exhaust dilution ratio was 18:1, resulting in a particle concentration of about 6 mg/m^3. The exhaust was generated by a Nissan CN-6 engine operated on the Federal Short Cycle. Characteristics of the exposure atmosphere are given in Table 4-9. Exposure to the diesel exhaust resulted in enhanced susceptibility to the lethal effects of *S. pyogenes* infection at all exposure durations (2 and 8 h; 8, 15, 16, 307, and 321 d). Tests with *Salmonella* were inconclusive due to the high mortality rates in the controls. The mice exposed to diesel exhaust exhibited no enhanced susceptibility to infection when challenged with the influenza virus.

TABLE 4-9. COMPOSITION OF EXPOSURE ATMOSPHERE IN CAMPBELL et al., STUDY[a]

	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (ppm)	HC (ppm)
Test	5.3-7.9	19-22	1.8-3.6	10-16	0.9-2.8	7-10
Control	—	1.2-2.7	0.02-0.13	0.03-0.17	0.04-0.94	2.9-4.7

[a]Range of mean values.

Source: Campbell et al., 1981.

Hatch et al. (1985) reported that *in vitro* tests on rabbit alveolar macrophages exposed to diesel exhaust resulted in significant decreases in viability and cellular ATP. In contrast, *in vivo* tests on CD-1 mice revealed no changes in susceptibility to *Streptococcus*

infection following intratracheal injection of 100 μg of diesel exhaust particles suspended in unbuffered saline. The recorded excess mortality was 14.4±11.3 percent (S.E), and not significantly different from zero. Hatch et al. (1985) suggested that any exhaust-induced effects on macrophage viability in the *in vivo* test might have been compensated for by other factors such as an increased influx of phagocytically active leukocytes from the blood.

NIOSH-Sponsored Studies. The effects of diesel exhaust on the immune system of male Fischer 344 rats were investigated by Mentnech et al. (1984). Exposures were for 7 h/d, 5 d/week for up to 2 yr. The diesel emissions were generated by a four-cycle, Caterpillar engine equipped with a water scrubber. The 24-mo mean concentration of particulate matter was 1.95±0.25 mg/m^3. As indicated by Lewis et al. (1986), the mass median aerodynamic diameter of the particles was 0.23 and 0.36 μm by the instrumental and scanning electron microscope techniques; the geometric standard deviations were 2.5 and 2.0, respectively. The 24-mo mean concentrations of other exhaust components were 11.5 ppm CO; 8.7 ppm NO; 1.5 ppm NO_2; 0.81 ppm SO_2; 60.2 ppb acrolein; and 38.3 ppb formaldehyde (as given in Lewis et al., 1986). The exposed animals were tested for immunocompetency by determining antibody-producing cells in the spleen 4 d after immunization with sheep erythrocytes. Proliferative response of splenic T-lymphocytes to the mitogens concanavalin A and phytohemagglutinin was used as the monitoring technique. There were no statistically significant differences between the test and control animals after 12 and 24 mo of exposure.

In related NIOSH-sponsored studies, the effects of diesel exhaust on interferon production and susceptibility of mice to viral infection was invesigated (Hahon et al., 1982, 1985). In *in vitro* studies using mammalian LLC-MK_2 cells, viral induction of interferon was depressed by about 60 percent following exposure of the cells to diesel exhaust particles (Hahon et al., 1982). Influenza virus growth in pretreated cell monolayers was 2 to 3 times higher than that in controls. In *in vivo* studies conducted on Ao/PR/8/34 influenza virus-infected CD-1 Swiss mice, 3- and 6-mo exposures to diesel exhaust resulted in significant increases in the percentage of animals showing lung consolidation, higher virus growth, depressed IFN levels, and a fourfold reduction in hemagglutinin antibody levels (Hahon et al., 1985). These effects were not seen after 1-mo exposure. The test animals

were exposed for 7 h/d, 5 d/week, and the exhaust was diluted to achieve a particle concentration of 2 mg/m^3.

Lovelace Studies. Bice et al. (1985) evaluated the development of the immune response in Fischer 344 rats and CD-1 mice exposed to diesel exhaust in the Lovelace inhalation toxicity studies. The test animals were exposed to three dilutions of diesel exhaust for 7 h/d, 5 d/week, for 30 mo. The three exhaust dilutions resulted in nominal particle concentrations of 0.35, 3.5, and 7 mg/m^3 (0.23, 0.25, and 0.26 μm MMAD, respectively). Concentrations of other exhaust components are given in Table 4-3. Test animals and controls were immunized by intratracheal instillation of sheep red blood cells (SRBC) after 6, 12, 18, and 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 (AFC) was elevated in rats, but not in mice, exposed to the two highest levels of diesel exhaust; after 6 mo of exposure, only the highest exhaust concentration caused this effect in rats. The number of AFC per 10^6 lymphoid cells in lung-associated lymph nodes and the level of specific IgM, IgI, or IgA in rat sera were not significantly altered. The investigators concluded that the exposures had only a minimal effect on the immune and antigen filtration function of the lymphoid tissue.

GM-Sponsored Studies. As part of the GM-sponsored series of studies on diesel exhaust, Dziedzic (1981) investigated the effects of exhaust on the number of T, B, and null lymphocytes in tracheobronchial lymph nodes and blood and spleen of male Hartley guinea pigs. The exhaust was generated with a 5.7-L, four-cycle, indirect injection engine operated at a steady speed and load (1350 rpm, 96 N•m), simulating 40 mph. The test fuel had a sulfur content of 0.27 percent. The pipes in the exhaust delivery system were heated to 100±15°C to simulate typical tailpipe conditions. The exhaust was then diluted with clean air (dilution ratio 40:1) to achieve a particle concentration of 1.533 mg/m^3 (0.2 μm MMAD) (Schreck et al., 1981). The diluted exhaust also contained 7.9 μg/m^3 CO and 9.2 μg/m^3 NO_x. The animals were exposed for 20 h/d, 5.5 d/week, for 4 and 8 weeks. There were no significant exposure-related changes in any of the measured parameters, and cell viability was comparable to the control value.

Other Studies. Takafuji et al. (1987) evaluated the IgE antibody response of BDF_1 mice innoculated intranasally at intervals of 3 weeks with varying doses of a suspension of diesel exhaust particles in ovalbumin solution. Antiovalbumin IgE antibody titres, assayed by passive cutaneous anaphylaxis, were enhanced by doses as low as 1 μg particles. Responses were higher than those in animals immunized with ovalbumin alone.

4.1.2.3.6. *Blood and Cardiovascular Systems*

Several studies have evaluated the effects of diesel exhaust exposure on hematological and cardiovasular parameters of laboratory animals.

EPA-Sponsored Studies. Wiester et al. (1980) studied the effects of diesel exhaust on the heart of male and female Hartley guinea pigs. The test animals were exposed 20 h/d for 28 d to irradiated and nonirradiated diesel exhaust containing 6 mg/m^3 of exhaust particles (other characteristics of the exposure atmospheres are given in Table 4-1). No effect on heart mass and no changes in ECG were observed. Animals exposed to irradiated exhaust did exhibit a small but significant decrease in heart rate.

NIOSH-Sponsored Studies. The effects of diesel exhaust on the cardiovascular system of Fischer 344 rats were investigated by Vallyathan et al. (1986) in NIOSH-sponsored studies evaluating the combined effects of diesel exhaust and coal dust exposure. The test animals (72 males and 72 females per test group) were exposed 7 h/d, 5 d/week for 2 yr. The exposure atmosphere contained 2 mg/m^3 of particulate matter (0.23 μm MMAD). The diesel emissions were generated by a four-cycle, Caterpillar engine equipped with a water scrubber. The fuel contained <0.5 percent sulfur by mass. As indicated by Lewis et al. (1986), the concentrations of other exhaust components were 11.5 ppm CO; 8.7 ppm NO; 1.5 ppm NO_2; 0.81 ppm SO_2; 60.2 ppb acrolein; and 38.3 ppb formaldehyde. Body weights, heart weights, right and left ventricular wall thickness, severity of cardiomyopathy, and changes in the small pulmonary arteries were evaluated. No statistically significant differences were seen in heart weight, heart wall thickness, or pulmonary artery wall thickness between control groups and those exposed to diesel exhaust alone, coal dust alone, or diesel exhaust and coal dust; however, there was a trend toward increased pulmonary arterial wall thickness in the diesel exhaust-exposed animals.

DOE-Sponsored Studies. As part of a study examining the combined effects of inhaled diesel exhaust and coal dust in male Wistar rats, Karagianes et al. (1981) measured hematological parameters in animals exposed to diesel exhaust alone. The test animals (6 per test group) were exposed 6 h/d, 5 d/week, for 4, 8, 16, or 20 mo. The exhaust was diluted to achieve a particle concentration of 8.3±2.0 mg/m^3. The concentration of CO was maintained at 50 ppm, and that of NO_2 at 4 to 6 ppm. The exposure had no effect on body weight, mortality pattern, hematocrit, or differential leukocyte count. A 3 to 5 percent increase in percent COHb saturation was observed in the exhaust-exposed animals.

GM-Sponsored Studies. As part of the GM-sponsored studies, Penney et al. (1981) examined hematological and cardiovascular parameters in Fischer 344 rats (32 per test group) and Hartley guinea pigs (about 36 guinea pigs per test group) exposed to diesel exhaust 20 h/d, 5.5 d/week, for 78 weeks. Particle concentrations were 0.25, 0.75, and 1.5 mg/m^3. At the highest exposure, the concentration of CO was 4.3 ppm and NO_x was 5.9 ppm (concentrations at other exposure levels and for other components were not reported). Morphometric analysis revealed no significant alterations in heart mass at any exposure level. In addition, hemoglobin, hematocrit, and RBC levels were not significantly different from control values in either species.

Fraunhofer Institut Studies. Heinrich et al. (1982, 1985) measured hematological parameters in female Syrian golden hamsters exposed to diesel exhaust. The test animals (48 per test group) were exposed 7-8 h/d, 5 d/week, to exhaust diluted to achieve a particle concentration of 3.9 mg/m^3 (0.1 μm MMAD). Concentrations of other exhaust components are given in Table 4-6. Blood analyses conducted after 29 weeks of exposure indicated a low erythrocyte count accompanied by an increase in erythrocyte volume and a reduced leucocyte count in the exhaust-exposed animals.

Ishinishi Studies. Hematological effects of diesel exhaust in male and female Fischer 344 rats was evaluated by Ishinishi et al. (1986b) as part of a long-term inhalation exposure study. The rats (64, 95, or 120 per test group) were exposed to diluted exhaust from light-duty (LD) and heavy-duty (HD) diesel engines. Exposures were 16 h/d, 6 d/week, for 30 mo. Nominal concentrations of particulate matter in the separate tests were 0.1, 0.4, 1, 2, and 4 mg/m^3. Exposure conditions and exhaust component concentrations are given in

Table 4-4. No significant changes in hematological parameters occurred in the first 24 mo of exposure. At 30 mo there were slight increases in red blood cell count, hemoglobin concentration, and hematocrit in some exposed groups but not in others (no other data reported). The platelet count was significantly decreased in males exposed to 4 mg/m^3.

CCMC Study. Brightwell et al. (1986) evaluated the hematological effects of filtered and unfiltered diesel exhaust on male and female Fischer 344 rats and Syrian hamsters. The animals (144 rats and 312 hamsters per test group) were exposed for five 16-h periods per week for 2 yr. The exhaust was generated with a 1.5-L engine operated under the U.S. 72 (FTP) test driving cycle. Three exhaust dilutions were tested, producing particle concentrations of 0.7, 2.2, and 6.6 mg/m^3. At the highest exposure the NO_x concentration was 8±1 ppm. and the CO concentration was 32±11 ppm (concentration of other gaseous pollutants and for the other exposure levels were not given). Rats exposed to the highest levels of unfiltered diesel exhaust exhibited a significant reduction in lymphocyte concentrations. Significant increases in red blood cell count, hemaglobin, hematocrit, white blood cell count, segmented neutrophils, and prothrombin time were also reported.

4.1.2.3.7. *Serum Chemistry*

As part of chronic inhalation exposure studies, Ishinishi et al. (1986b) examined the effects of diesel exhaust on serum biochemistry in male and female Fischer 344 rats. The rats (64, 95, or 120 per test group) were exposed to diluted exhaust from light-duty (LD) and heavy-duty (HD) diesel engines. Exposures were 16 h/d, 6 d/week, for 30 mo. Nominal concentrations of exhaust particles in the separate tests were 0.1, 0.4, 1, 2, and 4 mg/m^3. Exposure conditions and exhaust component concentrations are given in Table 4-4. Compared to control values, significant decreases were seen in the activity of cholinesterase and in the concentrations of free cholesterol and phospholipid in HD males exposed to 4 mg/m^3 (no other data given). The latter two parameters were also reduced in HD males exposed to 2 mg/m^3.

Brightwell et al. (1986) measured changes in serum biochemical parameters in male and female Fischer 344 rats and Syrian hamsters exposed to both filtered and unfiltered diesel exhaust. The animals (144 rats and 312 hamsters per test group) were exposed for

five 16-h periods per week for 2 yr. Three diesel particle concentrations were used; 0.7, 2.2, and 6.6 mg/m^3. At the highest exposure, the CO and NO_x concentrations were 32 ppm and 8 ppm, respectively (concentrations of CO and NO_x at the other two exhaust levels were not reported). Rats exposed to the highest levels of unfiltered diesel exhaust showed significant reductions in blood glucose level, total blood proteins, triglycerides, and cholesterol. Significant increases in blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were also reported. These changes were attributed to possible reduced kidney function, impaired liver function, and hypoxia. Kidney or liver damage was not confirmed by histological examination. In hamsters, some blood chemistry changes, such as an increase in gamma-glutamyl transpeptidase were thought to be suggestive of liver damage.

4.1.2.3.8. *Effects on Enzyme Activity*

Several studies have examined the effects of diesel exhaust exposure on tissue enzymes associated with the metabolism and possible activation of polynuclear aromatic hydrocarbons (PAHs). As part of the EPA studies on diesel exhaust, 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 CD Sprague-Dawley rats exposed to the exhaust. The test animals were exposed to the diluted exhaust (13:1 dilution ratio) 20 h/d, for 42 d. The exposure atmosphere, as reported in Lee et al. (1978), included 6.32 mg/m^3 of suspended particles, 2.19 ppm NO_2, 2.13 ppm SO_2, 15.7 ppm CO, and 15.6 ppm total hydrocarbons (as carbon). After 33 d, AHH activity (pmol/min/mg microsomal protein) was 0.28±0.16 in the prostate, 4.19±0.32 in the lung, 117.5±4.6 in the liver, and 1.54±0.23 in the testis. Except for the testis, these values were significantly higher ($p < 0.05$) than corresponding activity in control animals. Epoxide hydrase activity was not significantly different from control values for any of the organs tested.

In the NIOSH-sponsored diesel exhaust studies, Rabovsky et al. (1984) investigated the effects of chronic inhalation of diesel exhaust and coal dust on microsomal cytochrome P450-associated benzo[a]pyrene hydrolase and 7-ethoxycoumarin deethylase activities in rat lung and liver. Male Fischer 344 rats were exposed 7 h/d, 5 d/week, for 24 mo to diesel

exhaust consisting of 2 mg/m^3 particles, 10.5 ppm CO, 1.5 ppm NO_2, and 0.6 ppm SO_2. The exposure had no effect on B[a]P hydrolyase or 7-ethoxycoumarin deethylase activities in lung or liver. In related studies, Rabovsky et al. (1986) examined the effects of diesel exhaust on virally induced enzyme activity and interferon production in female Swiss CD-1 mice. The mice were exposed 7 h/d, 5 d/week, for 1 mo to diesel exhaust diluted to achieve a particle concentration of 2 mg/m^3. Following the exposure, the mice were innoculated intranasally with influenza virus, and changes in serum levels of interferon and activity levels of three liver microsome enzymes associated with the cytochrome P450 pathway were recorded. In the absence of viral innoculation, exposure to diesel exhaust had no effect on activity levels of the three liver microsome enzymes; however, following viral innoculation, the temporal pattern of enzyme activity was altered by the exhaust.

Effects of diesel exhaust on enzyme activities in male Fischer 344 rats were studied by Chen and Vostal (1981) and Navarro et al. (1981) as part of the General Motors diesel toxicity studies. Chen and Vostal (1981) measured the activity of aryl hydrocarbon hydroxylase (AHH) and the content of cytochrome P450 in lungs and liver of animals exposed by inhalation or intraperitoneal (i.p.) injection (of dichloromethane extracts of the particulate matter). In the inhalation exposures, the exhaust was diluted to achieve particle concentrations of 0.75 or 1.5 mg/m^3, and the exposure protocol was 20 h/d, 5.5 d/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 d. Inhalation exposures 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 diesel exhaust 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 content in lungs or liver following either inhalation exposure or i.p. treatment.

In related studies, Navarro et al. (1981), evaluated the effect of diesel exhaust exposure on hepatic and pulmonary microsomal enzyme activities in Fischer 344 rats. The same exposure regime was used (20 h/d, 5.5 d/week, for up to 1 yr), and the exhaust was diluted to achieve particle concentrations of 0.25 and 1.5 mg/m^3 (a few tests 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 hepatic metabolism of B[a]P. However, 1 yr of exposure to 0.25 and 1.5 mg/m^3 impaired the ability of lung microsomes to metabolize B[a]P (0.015±0.05 and 0.12±0.01 nmole/30 min/mg protein, respectively, vs. 0.32±0.05 nmole/30 min/mg protein for the controls $p < 0.05$).

4.1.2.3.9. *Effects on Behavior*

In EPA studies Laurie et al. (1978) and Laurie et al. (1980) examined behavioral alterations in adult and neonatal Sprague-Dawley rats exposed to diesel exhaust. Laurie et al. (1978, as reported in Laurie et al., 1980) reported that rats exposed 20 h/d, 7 d/week, for 6 weeks, to exhaust containing 6 mg/m^3 of particulate matter exhibited a significant reduction in adult spontaneous locomotor activity (SLA) and in neonatal pivoting (no other data given). In a follow-up to this study, Laurie et al. (1980) found that shorter exposures (8 h/d) also resulted in a reduction in SLA in adult rats. The exhaust contained 5.97 mg/m^3 of particulate matter, 19.2 ppm CO, 7.29 ppm hydrocarbons, 2.51 ppm NO_2, and 1.82 ppm SO_2. Laurie et al. (1980) conducted additional behavioral tests on adult rats that had been exposed during the neonatal period. For two of three exposure situations (20 h/d for 17 d postpaturition, or 8 h/d for the first 28 or 42 d postpaturition), significantly lower SLA was observed in the majority of the tests conducted on the adults after week 5. When compared with control animals, adult 15-mo-old rats that had been exposed as neonates (20 h/d for 17 d) also exhibited a significantly slower rate of acquistion of a bar pressing task to obtain food. The investigators noted that the evidence was insufficient to determine whether the differences were due to a learning deficit or to some other cause (e.g., motivational or arousal differences).

4.1.2.3.10. *Neurophysiological Effects*

Neurophysiological effects of diesel exhaust in male Sprague-Dawley rats were investigated by Laurie and Boyes (1980, 1981). Rat pups were exposed to a 18:1 dilution of diesel exhaust (6 mg/m^3 particle concentration) for 8 h/d and 7 d/week (total exposure period not reported). 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 elicted by a flash of light, were the end points tested. An increased pulse latency was reported for the diesel-exhaust-exposed rats, and this was thought to be due to a reduction in the degree of nerve myelinization.

4.1.2.4. Effects on Reproduction and Development

EPA-sponsored studies on diesel exhaust have included an evaluation of potential teratogenicity in rats and rabbits (Werchowski et al., 1980a, 1980b). Twenty albino Spague-Dawley rats were exposed in inhalation chambers to an atmosphere containing 10 percent diesel exhaust and 90 percent clean air (no other data given). Exposures lasted 8 h/d and were conducted on days 6 through 15 of gestation. There were no signs of maternal toxicity or decreased fertility. No malformations or other teratogenic effects were observed in 20-d-old fetuses (Werchowski et al., 1980a). In a second study, 42 New Zealand albino rabbits were exposed to the same exhaust concentration as rats on day 6 through 18 of gestation. The exposures were for 8 h/d. No adverse effects on body weight gain or fertility were seen in the animals exposed to diesel exhaust. No visceral or skeletal developmental abnormalities were observed in the fetuses (Werchowski et al., 1980a).

Several studies have evaluated the effect of diesel exhaust exposure on sperm morphology. Pereira et al. (1981a) reported a significant (p = 0.0024) 2.67-fold increase in sperm abnormalities in male Chinese hamsters exposed for 8 h/d for 6 mo to diesel exhaust (concentrations of particulate matter and gaseous components were not reported). In another study in which A/Strong mice were exposed to diesel exhaust (particle concentration 6.0 mg/m^3) for 8 h/d for 31 or 38 weeks, no significant differences were observed in sperm morphology between exposed and control animals (Pereira et al., 1981b). It was noted, however, that there was a very high rate of spontaneous sperm abnormalites in this strain of mice, and this may have masked any small positive effect. Quinto and DeMarinis (1984) reported a statistically significant and dose-related increase in sperm abnormalities in male (C57Bl/6×C3H)F_1 mice dosed intraperitoneally for 5 d with 50, 100, and 200 mg/kg of diesel particulate matter suspended in corn oil. A significant decrease in sperm number was seen at the highest dose, but testicular weight was not affected by the treatment.

4.2. COMPARISON OF HEALTH EFFECTS OF FILTERED AND UNFILTERED EXHAUST

In several chronic toxicity studies on diesel exhaust, the experimental protocol included exposing some of the test animals to filtered exhaust containing no particulate matter. Comparisons were then made between the effects caused by the whole, unfiltered exhaust and those caused by the gaseous components of the exhaust.

In Fraunhofer Institut studies, Heinrich et al. (1982) compared the toxic effects of whole and filtered diesel exhaust in female Syrian golden hamsters and female Wistar rats. The exhaust was generated with a 2.4-L Daimler-Benz engine operated at a constant load of 16 kW and a uniform speed of 2400 rpm. The diesel fuel contained 0.36 percent sulfur. The exhaust was diluted to achieve a particle concentration of 3.9 mg/m^3 (0.1 μm MMAD). The concentrations of the other components of the exposure atmospheres are given in Table 4-6. The exposures were for 7 to 8 h/d and 5 d/week. Rats exposed for 24 mo to either whole or filtered exhaust exhibited no significant changes in respiratory rate, respiratory minute volume, compliance, or resistance. In the hamster tests, histological changes (adenomatous proliferations) were seen in the lungs of animals exposed to both whole and filtered exhaust; however, in all groups exposed to the whole exhaust, the number of animals exhibiting such lesions was significantly higher than for the corresponding groups exposed to filtered exhaust or clean air. Both control and test animals exhibited increasingly serious degrees of amyloidosis of the kidneys, adrenals, liver, and spleen. In addition, cysts were observed in the liver of both control and treated animals.

In another Fraunhofer Institut study, Heinrich et al. (1986a, see also Stoeber, 1986) compared the toxic effects of whole and filtered diesel exhaust in hamsters, rats, and mice. The exhaust was generated by a 40-kW, 1.6-L engine operated under the U.S. 72 (FTP) test driving cycle. The fuel contained 0.36 percent sulfur. The exhaust was diluted to achieve a particle concentration of 4.24 mg/m^3 (0.35 μm MMAD). The concentration of the other components of the exposure chambers are given in Table 4-7. The test animals (96 per test group) were exposed for 19 h/d, 5 d/week, for 120 (hamsters and mice) or 140 (rats) weeks. Body weights of hamsters were not affected 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 yr of exposure to whole exhaust. After 1 yr 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 lungs; whole exhaust caused thickened alveolar septa, bronchiolo-alveolar hyperplasia, and emphysematous lesions, but no lung tumors. In mice, whole exhaust, but not filtered exhaust, caused multifocal bronchiolo-alveolar hyperplasia, multifocal alveolar lipoproteinosis, and multifocal interstitial fibrosis. The incidence of adenocarcinomas was significantly increased in both groups (the control group had a very high incidence rate for spontaneous tumors). In rats, there were no significant morphological changes in animals exposed to filtered exhaust and no increase in lung tumor incidence. In rats exposed to whole exhaust there were severe inflammatory changes in the lungs, thickened alveolar septa, foci of macrophages, crystals of cholesterol, hyperplastic and metaplastic lesions, and a significant increase in lung tumors (bronchiolo-alveolar adenomas and squamous cell tumors). Heinrich et al. (1986a) concluded that rats appeared to be more sensitive to the tumorigenic effects of diesel exhaust than mice and hamsters. Biochemical studies of lung lavage fluids of hamsters and rats indicated that exposure to filtered exhaust caused fewer changes than whole exhaust. The latter produced significant increases in lactate dehydrogenase, alkaline phosphatase, acid phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), 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 leucocytes (161±43.3/μL vs. 55.7±12.8/μL in the controls), a decrease in macrophages (30.0±12.5 vs. 51.3±12.5/μL in the controls), and a increase in granulocytes (125±39.7 vs. 1.23±1.14/μL in the controls). In all three cases the differences were significant at $p \leq 0.05$. There was also a slight increase in lymphocytes (5.81±4.72 vs. 3.01±1.23/μL in the controls).

In CCMC-sponsored studies, Brightwell et al. (1986) evaluated the toxic effects of whole and filtered diesel exhaust on male and female Fischer 344 rats and male and female Syrian hamsters. The exhaust was generated with a 1.5-L engine operated under the U.S. 72 (FTP) test driving cycle. Three exhaust dilutions were tested, producing particle concentrations of 0.7, 2.2, and 6.6 mg/m^3. At the highest exposure the NO_x concentration was 8±1 ppm, and the CO concentration was 32±11 ppm (concentrations of other gaseous pollutants and for the other exposure levels were not given). The test animals (144 rats and 312 hamsters per exposure group) were exposed for 5 16-h periods per week for 2 yr. The major histopathological finding was an increased incidence of lung tumors in rats exposed to the two higher levels of unfiltered diesel exhaust, as compared to controls (see Chapter 7). Rats exposed to the lowest concentration of particulate matter and those exposed to filtered diesel exhaust did not exhibit an increase in pulmonary tumors. Rats exposed to whole exhaust also exhibited marked obstructive and restrictive airway disease. There was no evidence of any toxicological effects in either rats or hamsters exposed to filtered diesel exhaust.

Iwai et al. (1986) exposed SPF Fischer 344 rats (24 per group) to whole or filtered diesel exhaust for 8 h/d, 7 d/week, for 24 mo. The whole exhaust was diluted to achieve a particle concentration of 4.9±1.6 mg/m^3. The exposure atmosphere also contained 30.9±10.9 ppm NO_x, 1.8±1.8 ppm NO_2, 13.1±3.6 ppm SO_2, and 7.0±1.4 ppm CO. Body weights in both exposed groups began to decrease after 18 mo while that of the controls did not. Lung-to-body weight ratios of the animals exposed to the total exhaust showed a significant ($p < 0.01$) increase after 12 mo in comparison to control values. Spleen-to-body weight ratios in both exposed groups were higher than control values after 24 mo. After 6 mo exposure to whole exhaust, particles accumulated in macrophages, and type II cell hyperplasia was observed. After 2 yr exposure, the alveolar walls had become fibrotic with mast cell infiltration and epithelial hyperplasia. In rats exposed to filtered exhaust, after 2 yr there were only minimal histologic changes in the lungs with slight hyperplasia and stratification of bronchiolar epithelia and infiltration of atypical lymphocytic cells in the spleen. Lung tumors occurred in animals exposed to whole exhaust, but not in those exposed to filtered exhaust. Malignant lymphoma of the spleen was the main cause of death in animals exposed to whole exhaust

and those exposed to filtered exhaust, and the incidence rate for both groups was significantly higher than that for the controls ($p < 0.05$).

4.3. INTERACTIVE EFFECTS OF DIESEL EXHAUST COMPONENTS

This section briefly discusses the potential toxicity of the various components of diesel exhaust and how they may interact to enhance or modify the overall effects of exposure to the exhaust. For more specific information the reader is referred to the following review and criteria documents. Health effects information concerning sulfur dioxide and particulate matter is available in an EPA Air Quality Criteria Document (U.S. EPA, 1982a) and in two addenda to that document (U.S. EPA, 1982b, 1986b). Information on acid aerosols can be found in an EPA Issue Paper (U.S. EPA, 1989a). Information on oxides of nitrogen is reviewed in a 1982 EPA Criteria Document (U.S. EPA 1982c). A revision of the Criteria Document is currently in the draft stage (U.S. EPA, 1989b). Health effects associated with carbon monoxide exposure are reviewed in an Air Quality Criteria Document (U.S. EPA, 1979) and in an addendum to that document (U.S. EPA, 1984b). A revision of the Criteria Document is currently in the draft stage (U.S. EPA, 1989c). Information on formaldehyde can be found in an EPA Health and Environmental Effects Profile (U.S. EPA, 1985), and information on acrolein in a Health Assessment Document (U.S. EPA, 1986a). Health effects information on polycyclic aromatic hydrocarbons is available in an EPA Drinking Water Criteria Document (U.S. EPA, 1987).

Case studies indicate that acute exposure to diesel exhaust can produce eye and respiratory tract irritation, headache, light-headedness, nausea, heartburn, numbness and tingling in extremities, chest tightness, and wheezing. Several components of the exhaust, such as NO_2, sulfuric acid, formaldehyde, and acrolein are irritants, and exposure to any one of these compounds, at sufficiently high concentrations, could result in burning and itching sensations in the eyes, nose, and throat, and may also produce wheezing and coughing. When these irritants are present together, as in a diesel exhaust, their combined effect may produce a response at concentrations below the thresholds for the individual compounds.

One physiological response to respiratory irritation is a reduction in the respiratory rate. According to Kane and Alarie (1978), the two major aldehyde components of diesel exhaust, formaldehyde and acrolein, affect the same respiratory receptor site and, therefore, exhibit competitive agonism in this regard. Sulfur dioxide also induces a reduction in respiratory rates; however, in the presence of acrolein, the effect may be altered or blocked, possibly due to the formation of an acrolein-bisulfite adduct (Kane and Alarie, 1979). Other components of diesel exhaust such as NO_2 and CO_2 may act as respiratory stimulants and thereby counteract the effects of the aldehydes and SO_2.

Diesel exhaust can affect pulmonary function as indicated by reductions in vital capacity, dynamic compliance, diffusing capacity, and forced expiratory flow and increases in airway resistance. Nitrogen dioxide, sulfur dioxide, sulfuric acid, and formaldehyde have been identified as bronchoconstrictors and one or more of these compounds may contribute to the increases in airway resistance and associated changes in lung function parameters.

Animals exposed to diesel exhaust develop deficiencies in pulmonary defense mechanisms. This may come about as a result of reduced mucociliary clearance, diminished effectiveness of alveolar macrophages, or reduced immunological competence. Several exhaust components can affect mucociliary clearance. Nitrogen dioxide levels as low as 1 to 2 ppm decrease ciliary beating in the respiratory epithelium, and a level of 4 ppm may affect the phagocytic ability of the alveolar macrophages. Animals exposed to NO_2 have exhibited an enhanced susceptibility to bacterial and viral infections. Sulfuric acid aerosols also affect pulmonary clearance mechanisms. Fine aerosols (4 μm) of sulfuric acid generally have an inhibitory effect on clearance rates; however, low concentrations (0.1 mg/m^3) of large particles (7.5 μm) accelerate clearance, particularly in the upper airways. Aliphatic aldehydes also cause reductions in mucociliary clearance.

Very limited data suggest that exposure to diesel exhaust may cause minor neurobehavioral effects such as decreased activity in laboratory animals. Of the components in diesel exhaust, carbon monoxide has been suspected of causing neurobehavioral effects as a result of interference with the oxygen-carrying capacity of hemoglobin. Another compound in diesel exhaust with similar properties is nitric oxide (NO). Carbon monoxide forms carboxyhemoglobin (CoHb) in the blood and NO forms nitrosylhemoglobin (NOHb)

which rapidly oxidizes to methemoglobin (MetHb). In diesel exhaust studies, concentrations of carbon monoxide have averaged 10 to 40 ppm, and those of nitric oxide 10 to 20 ppm. Studies on mice indicate that a continuous exposure to 10 ppm NO can result in NOHb and MetHb levels of 0.13 percent and 0.2 percent (Oda et al., 1976). In comparison, modeling studies indicate that 8-h exposures to 10 ppm CO may lead to 1 to 2 percent COHb in humans (U.S. EPA, 1984b). Carboxyhemoglobin levels of 5 percent and higher have been associated with neurobehavioral effects.

The histopathological changes seen in the lungs of animals exposed to diesel exhaust consist primarily of macrophage aggregation, alveolar epithelial Type II cell proliferation, fibrosis, and, in some cases, emphysematous-like changes. Histopathological effects reported for the various components of diesel exhaust vary in the sites and types of cells affected. Acute exposures to NO_2 result in death and desquamation of bronchiolar epithelial cells and Type I alveolar cells and proliferation of Clara cells and Type II epithelial cells. Chronic exposures can lead to bronchiolitis and emphysematous-like lesions in the lungs. Acute exposures to acid aerosols such as sulfuric acid result in bronchial and bronchiolar epithelial desquamation, alveolitis, and edema. Chronic exposures cause hypertrophy and hyperplasia of bronchial and bronchiolar epithelial secretory cells and may result in chronic bronchitis. Subchronic exposure to fine aerosols of ammonium sulfate (0.3 μm MMAD) have been reported to cause lung structural changes in hamsters. Of the aldehydes in diesel exhaust, formaldehyde induces hyperplastic lesions in the epithelium of the upper respiratory tract, and acrolein causes increased mucus secretion and bronchial epithelial proliferation which may lead to bronchitis and bronchiolitis.

The effects reported above may be altered in the presence of diesel particulate matter as a result of the adsorption of the compounds onto the particles. When adsorbed onto particles, the compounds can be transported and deposited deeper into the lungs, and being more concentrated on the particle surface, cytotoxic effects or physiological responses may be enhanced. Boren (1964) reported that NO_2 adsorbed onto carbon particles caused parenchymal lesions in mice, while the NO_2 alone produced edema and inflammation, but no lesions. Last et al. (1983) reported that collagen synthesis in lung tissue was higher in animals exposed to NO_2 and ammonium sulfate aerosols than in those exposed to NO_2

alone. Kilburn and McKenzie (1978) reported that exposure to formaldehyde and acrolein adsorbed onto carbon particles (1 to 4 μm) resulted in recruitment of polymorphonuclear leukocytes to tracheal and intrapulmonary epithelial tissue but not when the aldehydes were tested alone. In the case of SO_2, particulate matter acts as a catalytic surface increasing the rate of conversion of SO_2 to the more toxic sulfuric acid. The bronchoconstrictive effects of SO_2 are considerably enhanced in the presence of aqueous aerosols or particulate matter.

Adsorption of hydrocarbons, such as PAHs, onto diesel exhaust particulate matter could affect the bioavailability of these compounds. The size, effective surface area, and chemical composition of the particles, as well as the presence of other adsorbed compounds such as sulfuric acid, may have an effect on the rate of dissolution, metabolic degradation, or toxicological activity of the PAHs at the level of the lung macrophages or lung epithelium surface.

Evidence of the importance of the particulate matter in enhancing the toxic effects of diesel exhaust is shown by chronic animal studies comparing the toxicity of whole diesel exhaust with that caused by filtered exhaust (see Section 4.2). As indicated by pulmonary function parameters, biochemical and histopathological changes in lung tissue, and the occurrence of lung tumors, these studies demonstrated that animals exposed to whole diesel exhaust exhibit more severe responses or a greater incidence of adverse responses than animals exposed to filtered diesel exhaust.

4.4. COMPARISON OF THE EFFECTS OF DIESEL EXHAUST AND GASOLINE EXHAUST

Diesel and gasoline engines differ considerably in the composition of their exhaust. Diesel engines generally have higher levels of particulate matter while gasoline engines have higher levels of carbon monoxide (Table 4-10).

The concentrations of nitrogen oxides and hydrocarbons in the exhaust vary more with the size of the engine than with the type of engine. A greater proportion of the exhaust hydrocarbons in diesel exhaust is associated with the particulate matter.

TABLE 4-10. EMISSION RATES FOR DIESEL[a] AND GASOLINE ENGINES

Vehicle type	Particles (g/km)	CO (g/km)	NO_x (g/km)	Hydrocarbons (g/km)
Light-duty diesel	0.1-0.4	0.5-3	0.5-2	0.05-0.8
Heavy-duty diesel	0.5-4	5-50	3-20	0.9-6
Light-duty gasoline	0.001-0.004	1-3	0.2-1	0.08-0.5
Heavy-duty gasoline	0.004-0.2	10-200	1-11	2-20

[a]Federal test procedures, hot start.

Source: Cuddihy et al., 1981., as adapted by Cuddihy et al., 1984.

The different compositions of diesel and gasoline engine exhaust can result in different toxic effects, and several studies have been undertaken to evaluate these differences.

Brightwell et al. (1986) compared the toxic effects of filtered (FDE) and unfiltered diesel exhaust (DE), gasoline exhaust (GE), and gasoline exhaust from an engine equipped with a catalytic converter (CGE). The test animals, male and female Fischer 344 rats and Syrian hamsters, were exposed for 5 16-h periods per week for 2 yr. Three diesel particle concentrations were used: 0.7, 2.2, and 6.6 mg/m^3. At the highest diesel exposure, the CO concentration was 32 ppm and the NO_x concentration was 8 ppm (concentrations of CO and NO_x at the other two exhaust levels were not reported). The concentration of CO was 224 ppm in the GE test and 21 ppm in the CGE test. The concentration of NO_x was 49 ppm in the GE test and 7 ppm in the CGE test. The diesel-exposed rats exhibited alterations in respiratory physiology indicative of obstructive and restrictive airway disease (specific data not given). These changes were not seen in rats exposed to gasoline exhaust. Rats exposed to DE and FDE had elevated levels of RBC, hemoglobin, and hematocrit, suggestive of hypoxia. Gasoline exhaust-exposed rats had similar hematological changes as well as significantly enlarged hearts. These changes were thought to be due to hypoxia brought

about by high CO levels. The blood carboxyhemoglobin level was 20 percent. The major histopathological finding was an increased incidence of lung tumors in rats exposed to the two highest levels of unfiltered diesel exhaust. Rats exposed to the lowest concentration of particles, those exposed to filtered diesel exhaust and those exposed to the gasoline exhausts, did not exhibit an increase in pulmonary tumors. No other histological data were presented.

The EPA conducted a 5-yr long study on the toxic effects of gasoline engine exhaust on beagle dogs (Stara et al., 1980). The test animals were exposed to irradiated (I) or nonirradiated (R) exhaust with or without concurrent exposure to a mixture of sulfur dioxide and sulfuric acid. The exposures were for 16 h/d and 7 d/week. The composition of the exposure atmospheres is given in Table 4-11. Postexposure studies indicated that the exhaust-exposed animals had higher levels of hemoglobin and hematocrit. Red blood cell counts were also elevated, but not significantly. There were no effects on white blood cells or clinical chemistry parameters (Orthoefer et al., 1980), and no significant effects on lung collagen content (Bhatnagar, 1980). At 18 and 36 mo of exposure, pulmonary function parameters were not significantly altered in the exposed animals. After 61 mo, dogs exposed to irradiated exhaust had higher total expiratory resistances than controls (Lewis and Moorman, 1980). Some evidence of right ventricular hypertrophy was also observed in dogs exposed to irradiated exhaust. Studies conducted 2 yr after the exposures ended revealed deficiencies in pulmonary function in dogs exposed to the nonirradiated exhaust (Gillespie, 1980). These deficiencies included abnormalities in ventilatory values, airway resistance, blood-gas exchange, and lung volumes. Animals exposed to irradiated exhaust exhibited similar, but less severe, changes in pulmonary function. Histological studies revealed substantial atypical bronchiolar epithelial hyperplasia in both the R and I groups as well as in the groups also exposed to SO_x (Hyde et al., 1980). The hyperplastic lesions were derived from nonciliated epithelial cells. Also seen were aggregations of inflammatory cells in the distal regions of the terminal bronchioles. Data on the degree of peribronchiolar fibrosis was not presented. The R+SO_x group showed the greatest severity of bronchiolar hyperplasia, the highest level of pulmonary resistance, and a significant increase in squamous metaplasia in the trachea and bronchi.

TABLE 4-11. EXPOSURE ATMOSPHERE FOR EPA CHRONIC TOXICITY STUDY[a]

Exhaust	CO (ppm)	NO_2 (ppm)	NO (ppm)	SO_2 (mg/m^3)	Sulfates (ppm)	Acrolein (ppm)	Formal (ppm)	Hydrocarbons (ppm)
Control	4.9	0.04	0.04	0.03	—	—	—	2.7
Nonirrad. (R)	97.5	0.05	1.45	—	0.37	—	—	27.5
Irrad. (I)[d]	94.5	0.94	0.19	—	5.9	0.0198	0.57	23.9
$R+SO_x$	98.4	0.05	1.51	0.48	21.0[b]	—	—	27.4
I^d+SO_x	94.8	0.89	0.19	0.42	99.2[c]	—	—	23.9

[a]Mean values.
[b]0.09 mg/m^3 sulfuric acid.
[c]0.11 mg/m^3 sulfuric acid.
[d]O_x (as O_3) = 0.20 ppm.

Source: Malanchuk et al., 1980.

4.5. SUMMARY AND DISCUSSION

4.5.1. Toxic Effects of Diesel Exhaust on Humans

Symptoms of acute exposure to diesel exhaust include mucous membrane and eye irritation, headache, light-headedness, nausea, vomiting, heartburn, weakness, numbness and tingling in extremities, chest tightness, and wheezing. Exhaust odors can cause nausea, headache, loss of appetite, and psychological stress. In one study in which a bus engine was operated under varying load conditions, a dilution factor of 140 to 475 was needed to reduce the exhaust odor to a threshold level.

The effects of short-term exposures to diesel exhaust have been investigated primarily in occupationally exposed workers. In studies on underground miners, bus garage workers, dock workers, and locomotive repairmen, changes in respiratory symptoms and pulmonary function over the course of a workshift were generally found to be minimal and not

statistically significant. However, in one study of bus garage workers, an increased frequency of symptoms of cough, labored breathing, itching, burning and watering of the eyes, chest tightness, and wheezing was observed. In addition, in several other studies, reductions in forced vital capacity (FVC) and forced expiratory volume at 1 s (FEV_1) were measured in the exposed workers. The latter effects were, in some cases, attributed to nitrogen dioxide or to nitrogen dioxide adsorbed onto particulate matter.

Chronic effects of diesel exhaust exposure have been evaluated in epidemiological studies of occupationally exposed workers including miners, railroad yard workers, stevedores, and bus garage mechanics. In most cases the data have been insufficient to establish clear correlations between effects and exposure level or length of employment. Such correlations may be complicated by various factors including smoking habits, occurrence of allergies, frequency of virally induced respiratory problems, variations in exhaust composition and workplace ventilation, and the possible presence of other workplace air pollutants. In a few of these studies a higher prevalence of respiratory symptoms, primarily cough and phelgm, were observed among the exposed workers. These symptoms were usually not accompanied by significant changes in pulmonary function. However, reductions in FVC and FEV_1 were reported in a few studies. Reductions in FEF_{50} and FEF_{75} have also been reported. In only one case was there sufficient evidence to indicate that exposure to diesel exhaust was associated with chronic respiratory disease.

4.5.2. Toxic Effects of Diesel Exhaust on Animals

Animal studies on the toxic effects of diesel exhaust have involved acute, subchronic, and chronic exposure regimes. In acute exposure studies, toxic effects have been associated primarily with high concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. In short-term and chronic exposure studies, toxic effects have been associated with exposure to exhaust particulate matter. Toxic effects of diesel exhaust in various animal species are summarized in Tables 4-12 to 4-15. The data are arranged on the tables in general order of increasing concentration of the exhaust particulate matter. The data for short-term exposures (Table 4-12) indicate minimal effects on pulmonary function even at

exhaust concentrations sufficiently high to cause histological and cytological changes in the lungs. Exposures for several months or longer resulted in accumulation of particles in the lungs, increase in lung weight, increases in macrophages and leukocytes, macrophage aggregation, hyperplasia of alveolar epithelium, and thickening of the alveolar septa. Similar effects have also been observed in studies in which animals have been chronically exposed to diesel exhaust. In addition, chronic exposures have also resulted in reduced growth rates and altered pulmonary function. Exposures to diesel exhaust have also been associated with increased susceptibility to respiratory tract infections, neurological or behavioral changes, some hematological changes, and morphological alterations in the liver.

4.5.2.1. Effects on Growth

The data presented in Table 4-13 show that growth of laboratory animals can be impaired by chronic exposures to diesel exhaust, particularly when the exhaust dilution results in particle concentrations of 2 mg/m^3 or higher and the exposure periods are 16 or more h/d. In studies in which the daily exposures were only 6 to 8 h/d, no effects on growth were seen even at particle levels of 6 to 8 mg/m^3.

4.5.2.2. Effects on Pulmonary Function

Alterations in pulmonary function that have been observed in animals chronically exposed to diesel exhaust include decreases in vital capacity, residual lung volume, diffusing capacity, dynamic lung compliance, and expiratory flow rates, as well as increases in airway resistance (Table 4-14). These effects were not reported in all exposure studies or for all species tested. They generally appeared only after prolonged exposures. The lowest exposure levels (expressed in terms of the diesel particulate concentration) that resulted in impairment of pulmonary function occurred at 2 mg/m^3 in cynomolgus monkeys, 11.7 mg/m^3 in cats, 1.5 and 3.47 mg/m^3 in rats, and 4.24 and 6 mg/m^3 in hamsters.

4.5.2.3. Histopathological and Histochemical Effects

Histological studies have demonstrated that chronic exposure to diesel exhaust can result in adverse effects on respiratory tract tissue (Table 4-15). Typical findings include

TABLE 4-12. SHORT-TERM TOXICITY OF DIESEL EXHAUST TO LABORATORY ANIMALS

Species	Exposure Period	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, mouse, hamster	20 h/d 7 d/week 86-90 d	1.5	—	—	—	Increase in lung weight. Increase in thickness of alveolar walls.	Kaplan et al., 1982.
Rat, mouse	7 h/d 5 d/week 19 weeks	4.38	—	—	—	No effects on lung function. Increase in neutrophils and lung proteases. Macrophage aggregation.	Mauderly et al, 1981
Rat	20 h/d 5 5 d/week 9 weeks	6 0	—	—	—	Macrophage aggregation. Incr. in polymorphonuclear leukocytes. Type II cell proliferation. Thickened alveolar walls.	White and Garg, 1981
Guinea pig	20 h/d 7 d/week 56 d	6.32	17 4	2.3	2 1	Incr. in relative lung wt Macrophage aggregation Hypertrophy of goblet cells. Focal hyperplasia of alveolar epithelium	Wiester et al., 1980
Cat	20 h/d 28 d	6.4	14 6	2 1	2 1	Few effects on lung function. Focal pneumonitis or alveolitis.	Pepelko et al, 1980a
Guinea pig	20 h/d 7 d/week 28 d	6 83[a]	16 7	2.9	1 9	Increase in pulmonary flow resistance. Bradycardia	Wiester et al, 1980

[a]Irradiated exhaust.

TABLE 4-13. EFFECTS OF CHRONIC EXPOSURES TO DIESEL EXHAUST ON GROWTH OF LABORATORY ANIMALS

Species	Exposure period	Particles mg/m^3	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, guinea pig	20 h/d 5 d/week 106 weeks	0 25 0 75 1 5	2 7[b] 4 4[b] 7 1[b]	0 1[c] 0 27[c] 0 5[c]	- - -	Reduced body weight in rats at 1.5 mg/m^3.	Schreck et al , 1981
Rat, monkey	7 h/d 5 d/week 24 mo	2	11 5	1 5	0 81	No effects on growth No hematological effects	Lewis et al , 1986
Rat[a]	16 h/d 5 d/week 30 mo	0 11 0 41 1 08 2 31	1 23 2 12 3 96 7 10	0 08 0 26 0 70 1 41	0 38 1 06 2 42 4 70	Dose-dependent decrease in growth Decreased survival between 24 to 30 mo	Ishinishi et al , 1986b
Rat, mouse	19 h/d 5 d/week 24 mo	4 24	12 5	1 5	1 1	Reduced body weights Increased mortality in mice	Heinrich et al , 1986a
Rat	16 h/period 5 periods/week 24 mo	0 7 2 2 6 6	- - 39 7	- - 0 68	- - -	Growth reduced at 2 2 and 6 6 mg/m^3.	Brightwell et al , 1986
Rat	7 h/d 5 d/week 30 mo	0 35 3 47 7 08	2 9 16 5 39 7	0 05 0 34 0 68	- - -	No effect on growth or mortality rates	McClellan et al , 1986
Rat	6 h/d 5 d/week 20 mo	8 3	50	4-6	-	No effect on growth or mortality rates	Karagianes et al , 1981 Vinegar et al , 1981a
Hamster	8 h/d 7 days/week 6 mo	6 12	-	-	-	No effect on growth Significant increase in lung wt and lung/body wt ratio	Vinegar et al , 1981b

[a]Data for tests with light-duty engine Similar results with heavy-duty engine
[b]Estimated from graphically depicted mass concentration data
[c]Estimated from graphically presented mass concentration data for NO_x (assuming 90 percent NO and 10 percent NO_2)

TABLE 4-14. EFFECTS OF DIESEL EXHAUST ON PULMONARY FUNCTION OF LABORATORY ANIMALS

Species	Exposure period	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Reference
Rat	20 h/d 5.5 d/week 24 mo	1.5	7.0	—	—	Increase in FRC,[a] ER,[b] RV,[c] MEF_{40},[d] $MEF_{\dot{r}20}$ $FEV_{0.1}$[e]	Gross, 1981b
Rat	7 h/d 5 d/week 24 mo	2	11.5	1.5	0.8	No effect on pulmonary function.	Lewis et al., 1986
Monkey	7 h/d 5 d/week 24 mo	2	11.5	1.5	0.8	Significant decrease in FEF_{50}/FVC and FEF_{40}/TLC.[f]	Lewis et al., 1986
Rat	7-8 h/d 5 d/week 24 mo	3.9	18.5	1.2	3.1	No effect on minute volume, compliance, or resistance.	Heinrich et al., 1982
Rat	19 h/d 5 d/week 140 weeks	4.24	12.5	1.5	1.1	Decrease in dynamic lung compliance. Increase in airway resistance.	Heinrich et al., 1986a
Hamster	19 h/d 5 d/week 120 weeks	4.24	12.5	1.5	1.1	Significant increase in airway resistance. Decrease in lung compliance.	Heinrich et al., 1986a
Hamster	8 h/d 7 d/week 6 mo	6 12	— —	— —	— —	Decrease in vital capacity, RV,[c] and diffusing capacity. Increase in static deflation lung volume.	Vinegar et al., 1981a, b
Cat	8 h/d 7 day/week 2.25 years	6.3[g] 11.7[h]	20.2 33.3	2.7 4.4	2.1 5.0	Decrease in vital capacity, total lung capacity, and diffusing capacity after 2 yr.	Pepelko et al., 1980b, 1981; Moorman et al., 1985
Rat	16 h/period 5 periods/week 24 mo	0.7 2.2 6.6	— — 32	— — —	— — —	Adverse pulmonary effects at 6.6 mg/m^3 (no other data).	Brightwell et al., 1986
Rat	7 h/d 5 d/week 30 mo	0.35 3.47 7.08	2.9 16.5 39.7	0.05 0.34 0.68	— — —	Diffusing capacity, lung compliance reduced at 3.5 and 7 mg/m^3	McClellan et al., 1986

[a]Functional residual capacity.
[b]Expiratory reserve.
[c]Residual lung volume.
[d]Max. expiratory flow at 40 percent VC.
[e]Forced expiratory volume in 0.1 s.
[f]Total lung capacity.
[g]First year of exposure.
[h]Second year of exposure.

TABLE 4-15. HISTOPATHOLOGICAL EFFECTS OF DIESEL EXHAUST IN THE LUNGS OF LABORATORY ANIMALS

Species	Exposure period	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Rat, mouse, hamster	20 h/d 7 d/week 86 90 d	1 5	–	–	–	Increase in lung weight Increase in thickness of alveolar walls	Kaplan et al , 1982
Rat	7 h/d 5 d/week 24 mo	2	11 5	1 5	0 8	Multifocal histocytosis Inflammatory changes Type II cell proliferation Fibrosis	Lewis et al , 1986
Monkey	7 h/d 5 d/week 24 mo	2	11 5	1 5	0 8	Macrophage aggregation No fibrosis, inflammation, or emphysema	Lewis et al , 1986
Rat	16 h/d 5 d/week 30 mo	0 11 0 41 1 08 2 31	1 23 2 12 3 96 7 10	0 08 0 26 0 70 1 41	0 38 1 06 2 42 4 70	Type II cell hyperplasia and lung tumors seen at >0 4 mg/m^3. Lesions in trachea and bronchi	Ishinishi et al , 1986b
Hamster	7-8 h/d 5 d/week 120 weeks	3 9	18 5	1 2	3 1	60 percent adenomatous cell proliferation.	Heinrich et al , 1982, 1985
Rat	19 h/d 5 d/week 140 weeks	4 24	12 5	1 5	1 1	Thickened alveolar septa Macrophage aggregation Inflammatory changes Hyperplasia Lung tumors	Heinrich et al , 1986a
Hamster	19 h/d 5 d/week 120 weeks	4 24	12 5	1.5	1 1	Thickened alveolar septa Bronchioloalveolar hyperplasia. Emphysema	Heinrich et al , 1986a

TABLE 4-15. (continued)

Species	Exposure Period	Particles (mg/m^3)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	References
Mouse	19 h/d 5 d/week 120 weeks	4 24	12 5	1 5	1 1	Bronchiolo-alveolar hyperplasia Alveolar lipoproteinosis Fibrosis.	Heinrich et al, 1986a
Guinea Pig	20 h/d 5 5 d/week 24 mo	0 25 0 75 1 5 6 0	–	–	–	Minimal response at 0 25 and ultrastructural changes at 0 75 mg/m^3. Thickened alveolar membranes, cell proliferation, and fibrosis at 6 0 mg/m^3 Increase in PMN leukocytes at 0 75 mg/m^3 and 1 5 mg/m^3	Barnhart et al, 1982 Vostal et al, 1981, 1982
Rat	7 h/d 5 d/week 30 mo	0 35 3 47 7 08	2 9 16 5 39 7	0 05 0 34 0 68	– – –	Alveolar and bronchiolar epithelial metaplasia at 3.5 and 7 0 mg/m^3. Fibrosis at 7 0 mg/m^3 Inflammatory changes	McClellan et al, 1986
Rat	16 h/d 5 periods/week 24 mo	0 7 2 2 6 6	– – 12	– – –	– – –	Increased incidence of lung tumors (no other data)	Brightwell et al, 1986
Rat	6 h/d 5 d/week 20 mo	8 3	50	4-6	–	Macrophage aggregation Alveolar cell hypertrophy. Interstitial fibrosis. Emphysema	Karaglanes et al., 1981
Cat	8 h/d 7 day/week 2 yr	6 34 11.70	20 2 33 3	2.7 4 4	2 1 5 0	Macrophage aggregation. Bronchiolar epithelial metaplasia Type II cell hyperplasia Peribronchiolar fibrosis	Plopper et al, 1983 Hyde et al, 1985
Hamster	8 h/d 7 d/week 6 mo	6 12	–	–	–	Macrophage accumulation Thickened alveolar lining. Type II cell hyperplasia Edema Increase in collagen	Pepelko, 1982b

alveolar histiocytosis, macrophage aggregation, tissue inflammation, increase in polymorphonuclear leukocytes, hyperplasia of bronchiolar and alveolar Type II epithelial cells, thickened alveolar septa, edema, emphysema, and fibrosis. Lesions in the trachea and bronchi have also been observed in some studies. The exposure levels (exhaust particle concentrations) at which one or more of these effects occurred were 3.9, 4.24, and 6 mg/m^3 in hamsters, 1, 2, 2.2, 3.5, 4.24, 6.0, and 8.3 mg/m^3 in rats, 1.5 mg/m^3 in guinea pigs, and 4.24 mg/m^3 in mice. 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 (G6P-DH), increased synthesis of collagen, and release of inflammatory mediators such as leukotriene LTB and prostaglandin $PGF_{2\alpha}$.

While the overall laboratory evidence is that prolonged exposure to diesel exhaust particulate matter results in histopathological changes in the lungs of exposed animals, several studies have also demonstrated that histopathological changes do not occur when particle concentrations are low, even following lifetime exposures. These no-effect levels were reported to be 2 mg/m^3 for cynomologous monkeys, 0.7 mg/m^3 for rats, and 0.25 mg/m^3 for guinea pigs.

The histopathological effects of diesel exhaust particulate matter appear to be strongly dependent on relative rates of pulmonary deposition and clearance. Clearance of particles from the lungs is a multiphasic process involving phagocytosis by alveolar macrophages. At particle concentrations of 1 mg/m^3 or above, pulmonary clearance becomes reduced and focal aggregations of particle-laden macrophages appear in the lungs, particularly in the alveoli and peribronchiolar regions, as well as in the hilar and mediastinal lymph nodes. With prolonged exposures, the continued presence of aggregations of soot-laden macrophages appears to result in an inflammatory tissue response, septal thickening, epithelial proliferation, and fibrosis. The critical factor in this process appears to be the minimal exposure, in terms of the concentration of the particulate matter, as well as in the length and frequency of the exposure periods, at which focal aggregations of soot-laden macrophages occurs. This exposure level may vary from species to species depending on rate of uptake and pulmonary deposition, pulmonary clearance rates, the relative size of the macrophage population per unit of lung tissue, the rate of recruitment

of macrophages and leukocytes, and the relative efficiency of the mucociliary and lymphatic transport systems.

4.5.2.4. Effects on Defense Mechanisms

Several animal studies have indicated that exposure to diesel exhaust can reduce an animal's resistance to respiratory tract infections. This effect, which can occur even after only a few hours of exposure, does not appear to be caused by direct impairment of the lymphoidal or splenic immune systems; however, in one study, interferon levels and hemagglutinin antibody levels were adversely affected in the exposed animals.

4.5.2.5. Neurological and Behavioral Effects

Behavioral effects have been observed in a few studies of animals exposed to diesel exhaust. These effects include reductions in spontaneous locomotor activity (SLA), as well as diminished learning ability. Neurophysiological studies have shown possible impairment of nerve impulse transmission.

4.5.3. Interactive Effects

The physiological and toxicological effects of diesel emissions can be enhanced or altered as a result of the combined actions or interactions of the various exhaust components. Nitrogen dioxide, sulfuric acid, SO_3, and aliphatic aldehydes are chemical irritants, and one or more of these components may contribute to the eye and respiratory tract irritation observed in acute exposures to diesel exhaust. Several of these exhaust components, including NO_2, sulfuric acid, SO_3, and aliphatic aldehydes, are broncho-constrictors. Sulfur dioxide and aliphatic aldehydes cause reductions in respiratory rates, while NO_2 may cause an increase. On an individual basis, any of these compounds could be expected to affect pulmonary function. However, the responses seen in short-term exposure studies with diesel exhaust are minimal, suggesting that concentrations are too low or that there are interactive effects diminishing the response.

Impaired resistance to respiratory tract infections have been reported in some diesel exhaust studies. This may come about as a result of reduced mucociliary clearance, diminished effectiveness of alveolar macrophages, or reduced immunological competence. Nitrogen dioxide, acrolein, formaldehyde, sulfuric acid, and inorganic sulfates may contribute to this effect as a result of their immediate cytotoxic activity and the resulting impairment of the mucociliary clearance mechanism in the respiratory tract.

The histological and cytological changes seen in the lungs of animals exposed to diesel exhaust have been attributed primarily to the accumulation and aggregation of exhaust particles in lung macrophages. Other exhaust components, particularly NO_2, aliphatic aldehydes, and sulfuric acid are also known to cause histopathological effects in the lungs. Most effects are directed at the bronchial and bronchiolar epithelial cells, with resulting hypertrophy and hyperplasia. Exposures to NO_2 can also lead to emphysematous lesions which have also been reported in some diesel studies.

Adsorption of inorganic or organic compounds on diesel particulate matter may alter the chemical composition and toxicological effects of the exhaust. Sulfur dioxide and nitrogen dioxide can be adsorbed onto exhaust particles. The SO_2 could be catalytically converted to sulfuric acid. Attached to the exhaust particles, these substances may be carried deeper into lungs where they might have a more direct and potent effect on epithelial cells or on alveolar macrophages engulfing the particles. In addition, adsorption of these compounds may alter the acidity of the particles and thereby cause physiochemical changes affecting the hydrocarbon fraction of the particles. Binding of the hydrocarbons and, consequently, bioavailablity may be altered by changes in pH, and this may result in more direct toxicological activity at the level of lung macrophages or lung epithelium surface.

4.5.4. Comparisons with Gasoline Exhausts

Laboratory studies indicate that prolonged exposure to gasoline engine exhaust can result in histopathological changes in the lungs of exposed animals. These changes consist primarily of atypical bronchiolar epithelial hyperplasia causing, in some areas, partial occlusion of the bronchiolar lumina. Hyperplastic changes involving bronchiolar epithelial

tissue are also a characteristic feature of chronic exposures to diesel exhaust. However, in the latter case there can also be extensive involvement of Type II cells in the alveolar region. This suggests that the mechanisms involved in the lung response to the two types of exhaust are similar but that the target tissue may be slightly altered due to the different physical and/or chemical composition of the exhausts. The higher levels of particulate matter in diesel exhaust may allow for a greater concentration of the active components of the exhaust in the deeper regions of the lungs. The effectiveness of diesel exhaust in inducing these tissue responses might also be enhanced by the presence of other exhaust components. The sulfur dioxide and sulfuric acid in diesel exhaust are known to induce pulmonary hyperplastic lesions and these components may have additive or synergistic effects in exposures to diesel exhaust.

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5. MUTAGENICITY

Since 1978 over 100 publications have appeared in which genotoxicity assays have been used to analyze diesel emissions, volatile and particulate extracts, or individual chemicals found in diesel emissions. While most of the early papers dealt with the question of whether diesel particulates exhibited mutagenic activity in the system of choice, most of the studies in recent years have employed bioassays (most commonly *Salmonella* TA98 without S9) to evaluate: (1) extraction procedures, (2) fuel modifications, (3) bioavailability of chemicals from particulates, and (4) exhaust filters or other modifications and other variables associated with diesel emissions. The use of bioassay-directed fractionation and chemical analyses to identify genotoxic components of diesel exhaust has been reviewed by Schuetzle and Lewtas (1986). Some of these studies are discussed in other sections of this document that deal with chemical identification of diesel emissions, metabolism, and mechanisms of carcinogenesis. This section will deal primarily with the aforementioned qualitative genotoxicity studies. Also, because of the large number of reports, this discussion will focus on key references. Several symposia on the health effects of diesel emissions have been held, and the published proceedings (U.S. EPA, 1980; Lewtas, 1982; Ishinishi et al., 1986) and review articles (Claxton, 1983; Pepelko and Peraino, 1983; IARC, 1989) on various aspects of the mutagenicity of diesel exhaust contain detailed discussions of individual reports.

5.1. GENE MUTATIONS

Huisingh et al. (1978) demonstrated that dichloromethane extracts from diesel particulates were mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and without rat liver S9 activation. This first report contained data from several different fractions as well as particulates from different vehicles and different fuels. Similar results with diesel extracts from various engines and fuels have been reported

by a number of investigators using the *Salmonella* frameshift-sensitive strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981; Brooks et al., 1984). Similarly, mutagenic activity was observed in *Salmonella* forward mutation assays measuring 8-azaguanine resistance (Claxton and Kohan, 1981) and in *E. coli* mutation assays (Lewtas, 1983). Claxton (1983) has provided a thorough discussion of the early studies using the *Salmonella* system, especially relating to some of the factors, such as fuel, type of combustion, environmental and atmospheric conditions that modify the generation of mutagens in diesel emissions.

In that review Claxton also noted that at least 184 chemicals had been identified in diesel exhaust. Of these, 44 had been evaluated in at least one mutagenicity assay with 21 giving clear results. Indeed, a large number of the reported studies using *Salmonella* have focused on the mutagenic activity of individual chemical components of diesel exhaust particulates. Several investigators, generally using TA98 without S9 activation and expressing mutagenic activity as activity per distance driven or mass of fuel consumed, have estimated that the nitroarenes (both mono and dinitropyrenes) contribute a significant amount of the total mutagenic activity of the whole extract (Nishioka et al., 1982; Salmeen et al., 1982; Nakagawa et al., 1983).

More recently, Matsushita et al. (1986) tested particulate-free diesel exhaust gas and a large number of benzene nitroderivatives (some of which have been identified as components of diesel exhaust gas). The exhaust gas and 61 of the 94 chemicals were mutagenic--Most more active in TA100 and tending to be very active without S9.

Mitchell et al. (1981) reported mutagenic activity of particle extracts of diesel emissions in the mouse lymphoma L5178Y mutation assay. Positive results were seen both with and without S9 activation in extracts from several different vehicles, with mutagenic activity only slightly lower in the presence of S9. These findings have been confirmed in a number of other mammalian cell systems using several different genetic markers. Casto et al. (1981), Chescheir et al. (1981), Li and Royer (1982), and Brooks et al. (1984) all reported positive responses at the HGPRT locus in CHO cells. Morimoto et al. (1986) used the APRT and Ouar loci in CHO; Curren et al. (1981) used Ouar in Balb/c 3T3 cells. In all of these studies, mutagenic activity was observed without S9 activation. Liber et al. (1981) used

the thymidine kinase (TK) locus in the TK6 human lymphoblast cell line and observed induced mutagenesis only in the presence of rat liver S9 when testing a methylene chloride extract of diesel exhaust. Barfnecht et al. (1982) also used the TK6 assay to identify some of the chemicals responsible for this activation-dependent mutagenicity. They suggested that fluoranthene, 1-methylphenanthrene, and 9-methylphenanthrene could account for over 40 percent of the observed activity.

Morimoto et al. (1986) injected diesel particulates (2 to 4 g/kg) into pregnant Syrian hamsters and detected increased APRT mutants in embryo cells cultivated 11 d after injection. Both light-duty and heavy-duty tars were effective. Schuler and Niemeier (1981) reported that an 8-h inhalation exposure of *Drosophila* to a fivefold dilution of exhaust from a diesel engine did not result in an increase in sex-linked recessive lethals. Specific-locus mutations were not induced in (C3H×101)F_1 male mice exposed to diesel exhaust 8 h/d, 7 d/week for either 5 or 10 weeks (Russell et al., 1980). The exhaust was a 1:18 dilution, and the average particle concentration was 6 mg/m^3. After exposure, males were mated to T-stock females, and matings continued for the reproductive life of the males. The results were unequivocally negative; no mutants were detected in 10,635 progeny derived from postspermatogonial cells or in 27,917 progeny derived from spermatogonial cells.

5.2. CHROMOSOME EFFECTS

Mitchell et al. (1981) and Brooks et al. (1984) reported increases in sister chromatid exchanges (SCE) in CHO cells exposed to particulate extracts of emissions from both light-duty and heavy-duty diesel engines. Morimoto et al. (1986) observed increased SCE from both light-duty and heavy-duty tar samples in PHA-stimulated human lymphocyte cultures. Tucker et al. (1986) exposed human peripheral lymphocytes from four donors to direct diesel exhaust for up to 3 h. Cell cycle delay was observed in all cultures along with increased SCE levels in two of the four cultures. Structural chromosome aberrations were induced in CHO cells by particulate extracts from a Nissan diesel engine (Lewtas, 1983) but not by similar extracts from an Oldsmobile diesel engine (Brooks et al., 1984).

Pereira et al. (1981a) exposed female Swiss mice to diesel exhaust 8h/d, 5 d/week for 1, 3, and 7 weeks. The incidence of micronuclei and structural aberrations were similar in bone marrow cells of both control and treated mice. Increased incidence of micronuclei, but not SCE, were observed in bone marrow cells of male Chinese hamsters after 6 mo exposure to diesel exhaust (Pereira et al., 1981b).

Guerrero et al. (1981) observed a linear dose-related increase in SCE in lung cells cultured after intratracheal instillation of diesel exhaust particulates at doses up to 20 mg/hamster. However, they did not observe any increase in SCE after 3 mo of inhalation exposure to diesel exhaust particulates (6 mg/m^3).

Pereira et al. (1982) measured SCE in embryonic liver cells of Syrian hamsters. Pregnant females were exposed to 12 mg/m^3 diesel particulates for 9 d or injected with diesel particulates or extracts 18 h before sacrifice. No increased level of SCE was observed in the inhalation experiment. The authors reported that the injection of the extract but not the particulate resulted in a dose-related increase in SCE.

Russell et al. (1980) reported no increase in either dominant lethals or heritable translocations in males of T-stock mice exposed by inhalation to diesel emissions. In the dominant lethal test T-stock males were exposed for 7.5 weeks and immediately mated to females of different genetic backgrounds [T-stock; (C3H×101); (C3H×C57Bl/6); (SEC×C57Bl/6)]. There were no differences from controls in any of the parameters measured in this assay. For heritable translocation analysis, T-stock males were exposed for 4.5 weeks, mated to (SEC×C57Bl/6) females, and the F_1 males were tested for the presence of heritable translocations. No translocations were detected among 358 progeny tested.

5.3. OTHER GENOTOXIC EFFECTS

Pereira et al. (1981c) exposed males of strain A mice to diesel exhaust particulates for 31 or 39 weeks using the same exposure regimen as noted in the previous section. Analyses of caudal sperm for sperm head abnormalities were conducted independently in three separate laboratories. Despite clear differences in scoring criteria between the laboratories,

all were in agreement that the exposed incidence was not above the control level. Conversely, male Chinese hamsters exposed for 6 mo (Pereira et al., 1981b) exhibited almost a threefold increase in sperm head abnormalities. However, the control incidence in strain A mice was in excess of 9 percent but less than 0.5 percent in the Chinese hamsters.

5.4. SUMMARY

Extensive studies with *Salmonella* have unequivocally demonstrated direct-acting mutagenic activity in both particulate and gaseous fractions of diesel exhaust. The induction of gene mutations has been reported in several *in vitro* mammalian cell lines after exposure to extracts of diesel particulates. Dilutions of whole diesel exhaust did not induce sex-linked recessive lethals in *Drosophila* or specific-locus mutations in male mouse germ cells. Structural chromosome aberrations and SCE in mammalian cells have been induced by particulates. Whole exhaust induced micronuclei, but not SCE or structural aberrations, in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 mo. In shorter exposure (7 weeks), neither micronuclei nor structural aberrations were increased in bone marrow of female Swiss mice. Likewise whole diesel exhaust did not induce dominant lethals or heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

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6. METABOLISM AND MECHANISM OF ACTION IN DIESEL EMISSION-INDUCED CARCINOGENESIS

The metabolism and proposed carcinogenic mechanism of action of chemicals are intrinsically related. It is difficult to obtain an understanding of one without consideration of the other and, therefore, both of these processes are being considered in this chapter. Specifically, emphasis will be given to polycyclic aromatic hydrocarbons (PAH) and aldehydes. These components are of greatest concern due to their demonstrated or suspected activity as carcinogens and/or procarcinogens, and their universal occurrence in diesel emissions. It is also well known that PAH biological reactivity and carcinogenicity are dependent upon metabolic processes (reviewed by Conney, 1982). Relative to diesel exhaust carcinogenicity, discussions in this chapter are limited to B[a]P, 1-nitropyrene, several dinitropyrenes, and aldehydes, all of which are present in diesel emissions.

6.1. METABOLISM CONSIDERATIONS

Several long-term inhalation studies have provided evidence for carcinogenicity and tumorigenicity of whole diesel exhaust in animals (Heinrich et al., 1986; Iwai et al., 1986; Mauderly et al., 1987). Over 100 carcinogenic or potentially carcinogenic components have been specifically identified in diesel emissions, including various PAHs. The PAH compounds are adsorbed to the carbon core of the particulate phase of the exhaust, and, as described in this chapter and in Chapter 7, this particle association is important relative to the biological effects of these components. Among the PAH compounds identified from diesel exhaust are benzo[a]pyrene (B[a]P), dibenz(a,h)anthracene, pyrene, chrysene, and nitroaromatics such as 1-nitropyrene (1-NP), 1,3-dinitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene, all of which are mutagenic, carcinogenic, or implicated as procarcinogens or cocarcinogens (Stenback et al., 1976; Weinstein and Troll, 1977; Thyssen et al., 1981; Pott

and Stober, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988).

6.1.1. Metabolism and Disposition of B[a]P

It is generally recognized that B[a]P is an activation-dependent carcinogen with the activated metabolites forming covalent DNA adducts (Boyland, 1980). The reactions responsible for this activation are mediated by the cytochrome P-450 complex and are known to occur in multiple tissues and in different species. The activation proceeds through Phase I oxidative and hydrolytic reactions which result in the formation of the ultimate carcinogenic metabolite, B[a]P 7,8-dihydrodiol 9,10-epoxide. Specifically, B[a]P undergoes a mixed function oxidase (MFO)-mediated epoxidation to form B[a]P-7,8-oxide which, in turn, is subjected to an epoxide hydrolase-mediated hydrolysis resulting in the stereoisomeric diols, (+)-B[a]P 7,8-dihydrodiol and (-)-B[a]P 7,8-dihydrodiol. The diasterioisomeric forms of B[a]P 7,8-diol 9,10-epoxide are derived following another P-450-mediated reaction. This metabolic pathway is summarized in Figure 6-1.

Several studies have examined the carcinogenic potential of diesel exhaust by evaluating the metabolism and disposition of constituents such as B[a]P. The principle source of environmental B[a]P is its association with airborne particles such as those generated by diesel engines and coke ovens (U.S. EPA. 1985).

Mitchell (1982), subjected 24 male F344 rats to nose-only inhalation of ^{3}HB[a]P aerosol (500 μg/L of air) for 60 min. High levels of radiolabel were detected in the trachea, lungs and turbinates. A biphasic clearance was noted with $t_{1/2}$ values of 2 to 3 h and 25 to 56 h. Absorption from the lungs and systemic distribution was demonstrated by the presence of radiolabel in soft tissues such as the liver, kidney, gastrointestinal tract, spleen, brain, and testes. The majority of the radiolabel in these tissues was removed after 2 d and the major route of excretion was in the feces. The significance of this study is the demonstration of rapid absorption and systemic distribution of B[a]P

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Figure 6-1. Metabolic pathway for B[a]P and formation of ultimate carcinogenic intermediate.

Adapted from: Levin et al., 1980.

following inhalation exposure, the ramifications of which may include altered metabolism due to circumventing the first-pass metabolism effect of the gastrointestinal tract or liver.

Metabolism of intratracheally instilled B[a]P (1.0 μg) in strain A/J mice exposed to diluted diesel exhaust (8 h/d, 7 d/week for 9 mo) was reported by Tyrer et al. (1981). The radiolabel (^{14}C or ^{3}H) was rapidly distributed throughout the body within 2 h. The highest levels were detected in the lungs, liver, and gastrointestinal tract. Only trace levels were detected in the gastrointestinal tract 168 h after administration. A companion study (Cantrell and Tyrer, 1980) examined the effects of diesel exhaust exposure on *in vivo* B[a]P metabolism in the aforementioned mice. Homogenates of lung, liver, and testes were obtained from five mice sacrificed at 2, 24, or 168 h after B[a]P instillation. HPLC analysis detected free B[a]P, nonconjugated primary metabolites, and sulfate, glucuronide and glutathione conjugates. Based on the concurrence of primary and secondary metabolites in each tissue, B[a]P metabolism was verified in all three tissues. The major hepatic metabolite was 3-hydroxy-B[a]P. No significant difference in metabolite profiles was noted between the diesel exhaust-exposed and the control (clean air) mice with the exception that diesel exhaust-exposed mice retained greater levels of parent compound in the lungs suggesting that B[a]P was adsorbed to the exhaust particles.

Leung et al. (1985) studied the role of microsomes in the removal and metabolism of B[a]P from diesel exhaust particles. Hepatic and lung microsomal preparations were made from 3-methylcholanthrene-induced F344 rats. ^{14}Carbon-B[a]P was adsorbed to diesel exhaust particles (0.49 μCi/mg) and incubated with the microsomal preparations. Results indicated that both lung and liver microsomes were capable of removing B[a]P from the exhaust particles and that this capacity was dependent upon the lipid content of the microsomes. The microsomes metabolized free B[a]P to B[a]P-9,10-diol and B[a]P-7,8,9,10-tetrol but did not metabolize particle-associated B[a]P.

Bond et al. (1984), however, demonstrated metabolism of particle-associated B[a]P and free B[a]P by alveolar macrophages (AM). AM isolated from beagle dogs were incubated with 1 μM ^{14}C-B[a]P in solution or with 1 μM ^{14}C-B[a]P coated onto diesel exhaust particles obtained from the exhaust of an Oldsmobile engine operated on the U.S. EPA driving cycle. Incubations were conducted at 37°C in 5 ml of Dulbecco's modified essential

medium ($2.0x10^6$ cells/mL) for 6, 12, 24, 36, or 48 h. The B[a]P concentrations used were not cytotoxic nor were they rate limiting. B[a]P-9,10-diol and B[a]P-7,8-diol were identified in the culture media and B[a]P-7,8-diol and B[a]P-4,5-diol were detected in the cellular extracts. Additionally, small amounts of B[a]P phenols and B[a]P quiniones were detected in both the cells and the media. The total amount of metabolites from both the cells and media were increased with increasing incubation time up to 48 h. However, use of B[a]P in solution or B[a]P coated onto diesel exhaust particles did not alter the total amount of metabolites produced by the macrophages over a 24-h incubation period. AM-mediated metabolism of particle-associated B[a]P is especially relevant considering that macrophages are instrumental in sequestering and transporting diesel exhaust particulate matter in the lungs. Although this investigation points to the ability of the AM to metabolize B[a]P associated with diesel particles, Chen and Vostal (1982) have reported that aryl hydrocarbon hydroxylase in AM is decreased after *in vivo* exposure to diesel exhaust. Whether such diesel-associated decreases in AM enzymatic activity is counterbalanced by increases in the AM population size in response to diesel particle deposition (White and Garg, 1981) is unknown. Moreover, further studies are required to determine the metabolic activity of AM as a function of cellular burdens of diesel particles; preferably, these studies should be conducted using human AM inasmuch as metabolic pathways present may qualitatively and quantitatively differ among species. Although it is known that human AM contain aryl hydrocarbon hydroxylase (AHH) activity (McLemore et al., 1981), and that they can metabolize B[a]P (Harris et al., 1978), comparative studies of the AHH activities in rat, hamster, and human AM could contribute toward determining the relationship such activity may have on the development of lung tumors.

Even though the AM appear to contain the bulk of diesel particles deposited in the lung during chronic exposures, other cell type may also participate in the metabolic activation of carcinogenic agents. Significant metabolism of B[a]P by rat type II alveolar lung epithelial cells was reported by Bond et al. (1983). In this study a lung epithelial cell line (LEC) was shown to metabolize B[a]P to B[a]P-7,8-diol and B[a]P-9,10-diol, the latter accounting for 80 percent of the total B[a]P metabolites. Small quantities of glucuronide conjugates of B[a]P-7,8-diol and 9-hydroxy-B[a]P. Pre-exposure of the cells to diesel exhaust

particle extract, benz[a]anthracene or coal gas condensate increased rates of B[a]P metabolism two- to fivefold. It was also found that pretreatment of LEC with diesel exhaust particle extract produced a threefold increase in covalent binding of ^{14}C-B[a]P. Compared to the AM that were examined in the aforementioned study, the rat Type II cells showed approximately 10 times greater activity in their ability to metabolize B[a]P.

Under healthy conditions, the Type II pneumocytes represent about 12 to 16 percent of all cells in the alveolar region of mammalian lungs and account for approximately 4 to 9 percent of the cells in the lungs (Crapo et al., 1983). AM, on the other hand, account for approximately 4 to 9 percent of the cells in the alveolar region (Crapo et al., 1983). In terms of their relative availability and existing information on their relative abilities to metabolize B[a]P, the Type II epithelial cells may play an even more important role in metabolically activating PAH than the AM, assuming PAH as a substrate is available to them (e.g., extraction of PAH from diesel particles by AM and the subsequent release of PAH or metabolically susceptible metabolites of PAH at Type II sites). The Type II cell hyperplasia observed after the deposition of diesel particles, and other types of particles (White and Garg, 1981; Lee et al., 1986; Lee et al., 1988; Plopper et al., 1983), seemingly would favor a prominent role for these cells in producing activated PAH metabolites. Another cell type that may be importantly involved in the metabolism of PAH to ultimate carcinogens is the Clara cell. This lung cell type is relatively rich in chemical metabolizing enzymes. The pulmonary P-450 system, for example, is present in Type II cells but it is not as concentrated in this epithelial cell type as it is in the Type II and Clara cell (Boyd, 1984). It is worthy to note that bronchoalveolar adenomas following diesel exposure have been found to resemble both Type II and Clara epithelial cells (Mauderly et al., 1987). Like the Type II pneumocyte, the Clara cells are viewed as not being importantly involved in the phagocytosis of particles that deposit in the lung. As previously indicated, any metabolism of procarcinogens by these cells probably involves the preextraction of carcinogen(s) in the extracellular lining fluid (ELF) and/or in other endocytic cells.

It is evident from the preceding studies that B[a]P adsorbed to diesel exhaust particles that are deposited in the respiratory tract is readily distributed throughout much of the organism via absorption from the lung and transport by the mucociliary escalator to the

gastrointestinal tract. The present data base appears to support the contention that particle-associated B[a]P can ultimately be metabolized by AM and or Type II cells to reactive intermediates following deep lung deposition of these particles.

6.1.2. Metabolism and Disposition of 1-Nitropyrene

1-Nitropyrene (1-NP), a genotoxic and carcinogenic nitro-substituted PAH, is a particle associated component of diesel exhaust (Pitts et al., 1982; Schuetzle et al., 1982; King, 1988). As with B[a]P, several investigators have studied the metabolism and disposition of 1-NP both in free form and in association with diesel exhaust particles.

Bond and Mauderly (1984) quantitated 1-NP metabolism and covalent binding in the isolated-perfused rat lung. The study verified oxidation, reduction, acetylation, and conjugation biotransfomation of 1-NP by the lung with oxidation being the major process. The overall metabolism of 1-NP was increased by prior exposure of the rats to the MFO inducer 3-methyl cholanthrene (3-MC) but not to phenobarbital. Based on the absence of a concomitant increase in DNA-adducts with the increased covalent binding, it was uncertain if this covalent binding was due to formation of mutagenic, reactive intermediates.

A more recent study (Bond et al., 1986) examined the metabolism and deposition of free and particle-associated 1-NP in F344 rats. Results of the work indicated that the urinary and fecal excretion of ^{14}C-1-NP was not altered by exposure to the pure form or to that adsorbed on diesel exhaust particles. Pure 1-NP, possibly because of its smaller aerodynamic diameter, was more efficiently absorbed in the lung than was 1-NP coated onto diesel exhaust particles and, therefore, greater lung retention was noted for particle-adsorbed 1-NP. However, no significant difference between the two forms of 1-NP was noted for nonpulmonary tissue distribution or metabolic profiles. Analysis of excreta and tissues indicated that 1-NP is rapidly metabolized by the lungs or metabolized by other tissues following translocation from the lungs. For both 1-NP forms, small amounts of 6- and 8-hydroxyacetylaminopyrene were detected in the lungs suggesting pulmonary oxidation, reduction, and conjugation of the parent compound. The demonstration of pulmonary metabolism of 1-NP and greater retention of 1-NP when adsorbed to diesel exhaust particles

may be of significance regarding the dose to the lungs of both parent compound and metabolites.

Whole body exposure of rats to diesel exhaust (7.4 mg/m^3) 7 h/d, 5 d/week for 4 weeks resulted in twofold increases in the rates of nitropyrene metabolism in nasal tissue and in isolated perfused lungs from these animals (Bond et al., 1986). HPLC analysis of ethyl acetate-extractable 1-[^{14}C]NP metabolites indicated that the major metabolites were 3-, 6-, and 8-hydroxy-1-aminopyrene and 4,5-dihydro-4,5-dihydroxy-1-nitropyrene. Furthermore, a fourfold increase in ^{14}C covalently bound in the lungs of these rats was detected. The increase in 1-NP metabolism was not observed for rats of lower (0.35 or 3.3 mg/m^3) exposure groups or clean air controls. The data from this study indicate that exposure to diesel exhaust particulate matter at concentrations of 7.4 mg/m^3 significantly alters the metabolism and subsequent covalent binding of nitropyrene.

Ball and King (1985) administered [^{14}C]1-NP to rats intraperitoneally, orally or by intratracheal instillation of vapor-phase-coated diesel exhaust particles (380 μg [^{14}C]1-NP/g; 5 mg/rat). Over 59 percent of the radiolabel was recovered within 24 h regardless of the route of administration. The metabolic profile and elmination kinetics were similar for all routes of administration. Of the recovered radiolabel, 20 to 30 percent was in the urine and 40 to 60 percent was in the feces. The principle urinary metabolite (representing 15 to 25 percent of the total urinary ^{14}C) was 6-hydroxy-*N*-acetyl-1-aminopyrene (6-OH-NAAP), a compound with demonstrated S-9 dependent mutagenic activity in *Salmonella* strain TA98. Gut flora was shown to be necessary for the formation of 6-OH-NAAP and for the observed enterohepatic circulation of first pass hepatic metabolites. Accumulation of ^{14}C and diesel exhaust particles was detected in the lungs and gastrointestinal tract 24 h after intratracheal adminsitration, thereby attesting to the importance of mucociliary transport and distribution of particles and their adsorbed components. Based on these results and previous *in vitro* studies (King et al., 1983), demonstrating 1-NP binding to lung macromolecules, the authors note the possible risk to these organs relative to 1-NP. A proposed pathway for *in vivo* biotransformation of 1-NP is presented in Figure 6-2.

Howard et al. (1986) studied the binding of intratracheally instilled nitropyrenes and B[a]P to mouse lung DNA following preexposure to intratracheally instilled doses of the

putative inducing agents, B[a]P, diesel exhaust dichloromethane extract, or 1-NP. The results indicated 1-NP to be a potent DNA binding agent even in the absence of enzyme induction and that this potency was increased following B[a]P exposure. Dinitropyrene (a mixture of the 1,3-, 1,6-, and 1,8- isomers) was also a potent lung DNA binding agent with and without the inducers. B[a]P was not as potent a binding agent. Preexposure to the diesel exhaust extract but not to B[a]P resulted in increased DNA binding of B[a]P. Pretreatment with the dichloromethane extract of diesel exhaust failed to increase the DNA binding of the nitropyrenes. The significance of this report is the demonstration that exposure to diesel exhaust may potentiate the DNA binding of some of its components.

King (1988) provided information relating the metabolism of nitropyrenes and their carcinogenic potential. Briefly, dinitropyrenes were found to be much more carcinogenic than 1-NP. The cytosolic enzymes of the rat mammary gland activated dintiropyrenes by monoreduction to hydroxylamine followed by O-acetylation. Reduction and acetylation pathways for dinitropyrenes and subsequent DNA adduct formation were detected in intact cells. However, the intact mammary cells metabolized 1-NP primarily through oxidative pathways.

6.2. CARCINOGENIC MECHANISM OF PAH COMPONENTS OF DIESEL EXHAUST

The mechanism of action of known PAH carcinogens has been attributed to the reactivity of certain metabolic intermediates with cellular macromolecules and the subsequent formation of DNA adducts. As described in Section 8.5, B[a]P and 1-NP adsorbed to diesel exhaust particles can become available for biotransformation to known reactive intermediates, and macromolecular binding of these metabolites has been demonstrated.

The available data base does not allow for a definitive discussion of the carcinogenic mechanism action for these compounds as it specifically relates to diesel exhaust, but rather must be addressed from the standpoint of the chemicals *per se*. These data are derived primarily from *in vitro* studies that were not specifically concerned with the potential

Figure 6-2. Possible metabolic pathways for *in vivo* biotransformation of 1-NP to 6-hydroxy-*N*-acetyl-1-aminopyrene.

Source: Ball and King, 1985

carcinogenicity of diesel exhaust but are relevant because the compounds of concern are known components of diesel emissions.

6.2.1. Carcinogenic Mechanism of B[a]P

As a result of PAH metabolism studies such as those conducted on B[a]P, theories have been proposed regarding the molecular mechanism by which activated intermediates express their genotoxic effects. B[a]P served as the model for the "bay-region" concept summarized by Jerina et al. (1980). This proposed mechanism would also be applicable to such compounds as benz[a,h]anthracene which is a potent carcinogen also known to occur as a diesel exhaust combustion product.

Briefly, this concept states that compounds derived from an angular benz[a]anthracene nucleus may undergo epoxidation and if the resulting epoxide is located in the "bay region" will be better alkylating agents and, therefore, have greater genotoxic potential. The chemical reactivity of these "bay region" epoxides is positively correlated with biological reactivity of these compounds.

Based on the assumption that DNA adduct formation is a critical step in the initiation of carcinogenesis (Harris, 1985), increases in adducts provide a plausible mechanism by which association with particles might increase the carcinogenicity of organic compounds in long-term inhalation studies. Various studies have examined this process in relation to particle-associated B[a]P.

An experiment was undertaken to test the hypothesis that inhalation of B[a]P associated with carbon black (CB) particles would increase the levels of DNA adducts compared to inhalation of pure B[a]P (Wolff et al., 1989). DNA modification was measured using the ^{32}P-postlabeling method recently developed by Randerath et al. (1985). The high sensitivity of this technique ≈ 1 adduct in 10^{10} bases (Reddy and Randerath, 1986) allowed measurement of the low levels of DNA adducts resulting from repeated inhalation exposures to ^{14}C-B[a]P aerosols (2 mg/m^3), ^{14}C-B[a]P (2 mg/m^3) adsorbed to carbon black particles (97 mg/m^3) (B[a]P/CB), or filtered air. Total ^{14}C levels in the lung, an indicator of reactive B[a]P

metabolites, were 100-fold greater following exposure to B[a]P/CB than following exposure to B[a]P alone.

The levels of total DNA adducts in the lung were *not* significantly different whether the rats were exposed to pure B[a]P or B[a]P/CB. Exposure to B[a]P appeared to be the dominant factor in producing DNA damage with little influence of adsorption of B[a]P onto CB particles in terms of total DNA adducts and the B[a]P diol-epoxide(BPDE)-DNA adduct. However, association of B[a]P with CB resulted in the formation of lung adducts that were not seen in DNA from lungs of rats exposed to pure B[a]P. These adducts were not identified. It is possible that these adducts seen only in the B[a]P/CB exposures may play an important role in the potential tumorigenic effect of particle-associated B[a]P. Possible reasons for the discrepancy between particle effects on total DNA adducts and retention of ^{14}C include the possibility that the kinetics for formation and decline of DNA adducts are different than those of total bound ^{14}C. As a consequence, long-term retention of total B[a]P and metabolites in the lung may not be a good marker for genotoxic damage.

There were clear differences in the kinetics of the buildup and decline of DNA adduct levels and total ^{14}C for rats exposed to B[a]P/CB. The half-time for the decline of total ^{14}C was approximately tenfold greater than that for the decline in levels of DNA adducts for rats exposed to B[a]P/CB. Previous work has shown that at 1 d or later after the end of single exposures to B[a]P or B[a]P/CB, most of the ^{14}C present was bound to total macromolecules (Sun et al., 1988), presumably largely, non-DNA protein. This information in combination with the present data thus suggest that decline or repair of DNA adducts is considerably faster than that of protein turnover. This effect would lead to increased buildup of ^{14}C in the lung relative to DNA adducts following repeated exposures, as was observed. The half-times for decline in DNA adducts observed in the present work are similar to the half-times of approximately 4 weeks reported for B[a]P metabolite-DNA adducts in the lungs of A/HeJ and C57Bl/6J mice (Stowers and Anderson, 1985). Protein turnover is generally on longer time scales.

It appears that long-term retention of ^{14}C radiolabel in the lung may not be as important as previously suspected, at least with respect to indicating DNA damage. ^{14}C binding levels and DNA adducts were not closely related and it is clear from these results

that DNA adduct levels cannot be predicted form total ^{14}C levels. This observation is consistent with the work of Morse and Carlson (1985) who observed that binding levels of ^{3}H with lung protein were greater than levels of ^{3}H to lung DNA 6 h after administration of oral H-B[a]P to mice. They also found that ^{3}H binding to protein was more persistent than ^{3}H bound to DNA.

Caution should be used in interpreting the results from the present experiment in terms of possible implications for long-term exposures when carcinogenicity might be observed. The present experiment was of short duration (12 weeks); the same pattern of results might not continue after many months of exposure.. The adduct levels were higher in the rats exposed to B[a]P/CB than B[a]P after 12 weeks of exposure and so it is possible that this difference might become greater with continued exposure. In addition, the different adduct patterns between the B[a]P/CB and B[a]P exposures may indicate that other adducts, besides the BPDE-DNA adduct, are important in potential carcinogenic effects of B[a]P/CB exposures. Another factor to consider is the possible influence of a chronic inflammatory response, cell injury and cell proliferation which accompanies long-term exposures to inhaled insoluble particles (Morrow, 1986). Such responses are generally greater after prolonged exposure than those in the present 12-week exposure. These responses might be factors in progression to tumors in long-term inhalation exposures of rodents when large lung burdens of particles accumulate (Morrow, 1986), and in the increased incidence in tumors when B[a]P is merely mixed with Fe_2O_3 particles as opposed to being adsorbed onto the particle (Saffioti et al., 1965).

The potential interaction of diesel exhaust components may also be of concern regarding carcinogenicity. Laskin et al. (1976) (as cited in Lippmann, 1980) reported that SO_2 can act as a cofactor in the development of bronchial carcinomas. Compared to B[a]P exposure alone, combined SO_2 and B[a]P exposures resulted in a significant increase in the incidence of squamous cell carcinomas in rats.

6.2.2. Carcinogenic Mechanism of Nitropyrenes

Subcutaneous injection (4.0 mg total dose) of 1,3-NP, 1,6-NP, or 1,8-NP to male F344 rats resulted in a 100 percent (10 of 10) sarcoma incidence at the injection site. After 320 d observation, the dimethyl sulfoxide controls exhibited a tumor incidence of 0 percent (0 of 20). No tumors were found in rats receiving 4- or 40 mg 1-NP. Transforming activity was observed for the DNA from 4 of 11 dinitropyrene-induced tumors suggesting oncogene activation (Sato et al., 1986).

The absence of tumor formation by 1-NP and the positive results for the dinitropyrenes from the previous study is consistent with the findings of King (1988). In this study, female CD and F344 rats were adminstered 1.3-, 1.6-, or 1,8-NP, or 1-NP subcutaneously, intraperitoneally or intragastrically. Based on incidences of malignant histiocytomas, mammary gland tumors and leukemias, the dinitropyrenes were much more carcinogenic than 1-NP. Specifically, the potency order was: 1,6 > 1,8 > 1,3 dinitropyrene > 1-NP. Oral intubation was relatively ineffective in tumor induction by these compounds. Cytosolic enzymes of the rat mammary gland were capable of activating the dinitropyrenes to reactive intermediates that formed tRNA adducts. The adduct formation was of the same relative order as the carcinogenic potential and was catalyzed by rat mammary cytosol. In addition to demonstrating the potential of dinitropyrenes to induce three tumor types in rats, this study provided data affirming the importance of metabolic activation of the dinitropyrenes, the quantitative variability among these isomers relative to their carcinogenic activity, and the susceptibility of a tissue distant from the site of administration to the carcinogenic potential of these compounds following biotransformation. The data support the conclusion that the dinitropyrenes (especially 1,6- and 1,8-NP) are reduced by mammalian enzymes to reactive hydroxylamines which are in turn activated by acetyl CoA to potential carcinogens.

Maher et al. (1988) examined the metabolism of 1-NP and dinitropyrenes in cultured human fibroblasts. The results of their experiments using these diploid cells indicated that 1-NP underwent bioactivation to a form that produced stable covalent DNA adducts. The mutagenic effect of 1-NP was correlated with the cytotoxic effect which in turn correlated

directly with the number of DNA adducts. It was also reported that 1-nitrosoaminopyrene (1-NOP), a major metabolite of 1-NP was similar in its mutagenic response and extent of DNA adduct formation to 1-NP. The number of 1-NP or 1-NOP adducts per 10^6 DNA nucleotides to reduce survival of the normal (cells that were not repair deficient) cells to 37 percent was 25. However, comparisons with repair-deficient cells did indicate that nucleotide excision repair protected against the mutagenic and cytotoxic effects of both 1-NP and 1-NOP. The mutagenic potency of both 1-NP and 1-NOP was intermediate between that of N-acetoxy-2-acetylaminofluorene and benzo[a]pyrene-7,8-diol-9,10-epoxide. Based on *Salmonella typhimurium* assays, Heflich et al. (1985) reported that the mutagenicity of 1-NOP was 20-fold greater than that of 1-NP. However, if the number of DNA adducts formed by 1-NP or 1-NOP were considered, the frequency of mutation was similar for both compounds. A proposed pathway for cellular activation of 1-NP and DNA adduct formation is presented in Figure 6-3.

El-Bayoumy et al. (1988) investigated the comparative tumorigenicity of 1-NP and its reduced derivatives, 1-nitrosopyrene and 1-aminopyrene. Results of their tests using Sprague-Dawley rats indicated that 1-NP was considerably more carcinogenic than its reduced derivatives relative to production of mammary adenocarcinomas following administration of these compounds by gavage. These results appear to be in conflict with those of Wislocki et al. (1986) where 1-nitrosopyrene produced a greater incidence of hepatic tumors in newborn mice. However, the possibility of complete reduction of 1-NP to 1-aminopyrene by gastrointestinal and/or hepatic metabolism prior to its reaching the mammmary glands could explain this discrepancy.

Djuric et al. (1988) also provided data affirming the correlation between DNA binding of 1-NP or 1,6-dinitropyrene and the relative tumorigenicity of the two compounds. Following i.p. injections of the radiolabeled test compounds, covalent DNA binding was not detected for 1-NP treated animals but the DNA adduct, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene was detected in the livers, kidneys, urinary bladder, and mammary glands of rats given 1,6-dinitropyrene. Induction of nitroreductases failed to increase the DNA binding by 1,6-dinitropyrene suggesting that additional factors such as O-acetylation may affect the observed DNA binding. This and the other described studies emphasize the importance of

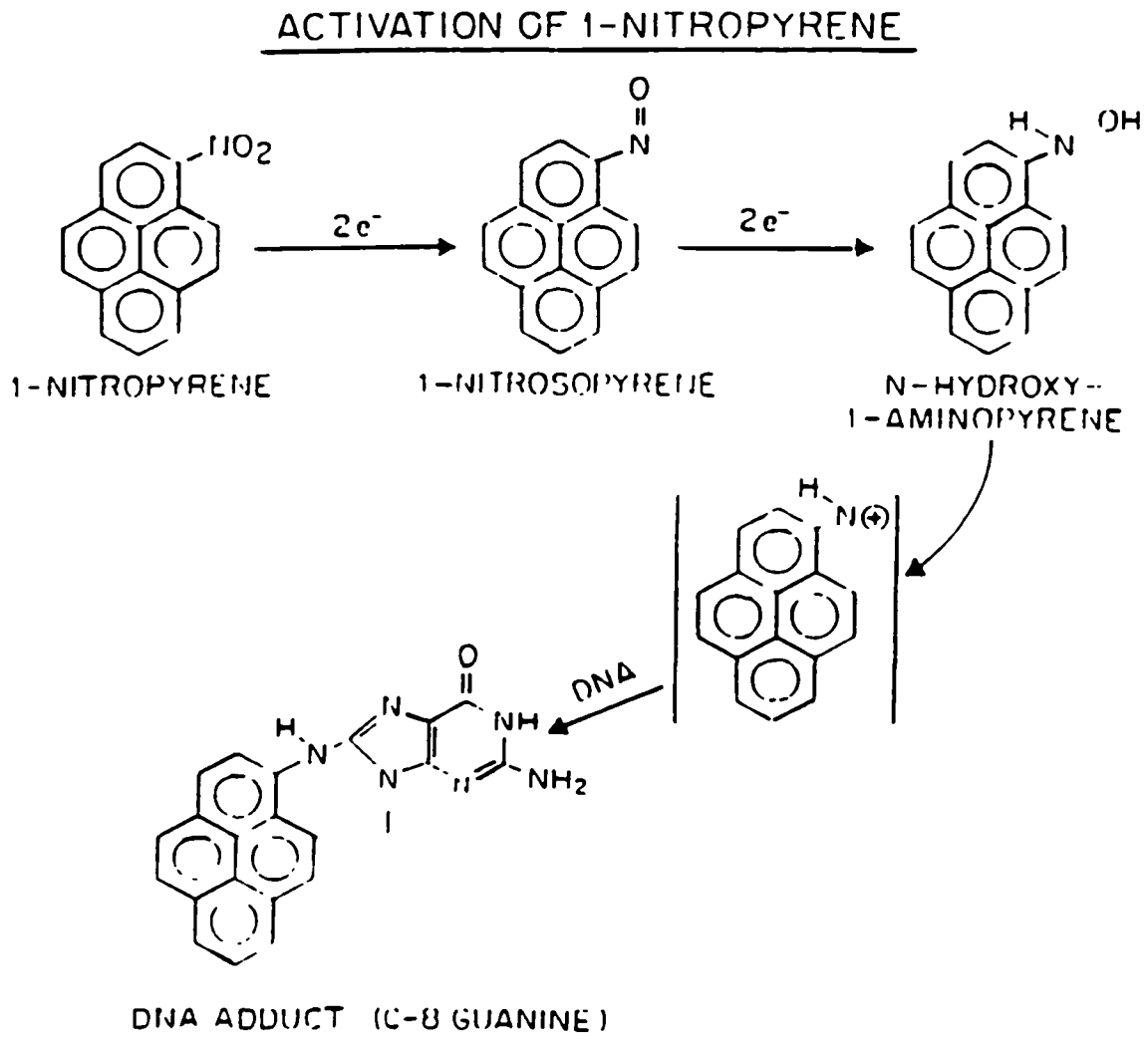

Figure 6-3. Proposed pathway for cellular activation of 1-nitropyrene (1-NP).
Source: Maher et al., 1988.

metabolism in determining the formation and subsequent carcinogenic activity of nitro PAH-nucleotide adducts.

6.3. CARCINOGENICITY OF ALDEHYDES

Regarding the carcinogenic potential of diesel emissions, most emphasis has been placed on the PAH components. However, various aldehydes, including acrolein and formaldehyde, are also components of diesel emissions. In addition to being primary irritants, both formaldehyde and acrolein are known carcinogens. Although the aldehydes are primarily gas-phase components of diesel emissions, they will be given consideration in this chapter.

6.3.1. Metabolism and Carcinogenicity of Formaldehyde

The pharmacokinetics of formaldehyde are reviewed in U.S. EPA (1985). Formaldehyde is readily absorbed in the upper and lower respiratory tract, and via most other routes of exposure. The metabolism of formaldehyde is complex due to its natural occurrence in the body, but is basically subjected to oxidation by aldehyde oxidase and conjugation with glutathione. The major end-products of formaldehyde metabolism are formic acid and CO_2, the production of these products having been verified in a wide variety of tissues and species.

Inhalation exposure to formaldehyde at 8 and 15 ppm produced tumors of the nasopharynx in rats (Clary et al., 1983). Similarly, clear evidence of carcinoma development in the nasal cavity and upper trachea was seen following inhalation exposure of rats to 14.3 ppm formaldehyde for 104 weeks (Kerns et al., 1983). A study by Albert et al. (1982) also indicated a significant increase in squamous cell carcinomas in rats discontinuously exposed to 14.1 to 14.3 ppm formaldehyde for 588 d. Other inhalation studies (Horton, et al., 1963; Dalbey, 1982) were also negative with respect to carcinogenic effects.

Epidemiologic studies regarding increased cancer incidence from formaldehyde exposure have not provided definitive findings. An analysis of carcinogenic risk to humans from exposure to formaldehyde is provided by Squire and Cameron (1984).

The carcinogenic mechanism of formaldehyde is unclear. Interactions with nucleic acids and other proteins has been demonstrated by several studies. The formation of reversible adducts and irreversible crosslinks with nucleic acids and proteins were detected by several investigators (French and Edsall, 1945; Feldman, 1973). The formation of DNA adducts and DNA-protein crosslinks in the nasal mucosa of rats was reported by Heck and Casanova-Schmitz (1983). Casanova-Schmitz et al. (1984) also found covalent binding to DNA in the olfactory mucosa of rats exposed to formaldehyde. More recently, Grafstrom et al. (1987) demonstrated formaldehyde-induced DNA single strand breaks and DNA protein crosslinks in cultured human bronchial cells.

6.3.2. Metabolism and Carcinogenicity of Acrolein

Definitive studies regarding the *in vivo* metabolism of acrolein are lacking. *In vitro* studies indicate that acrolein is metabolized by oxidative mechanisms to acrylic acid or glycidaldehyde (Patel et al., 1980), which may be followed by glutathione conjugation. Adduct formation with nucleic acids and proteins has been demonstrated (Chung et al., 1984). Acrolein-induced DNA protein crosslinking as a result of covalent binding has also been reported (Lam et al., 1985), and Munsch et al. (1974) provided *in vivo* data for covalent binding of acrolein to RNA, DNA, and protein in rat liver. Generally, sufficient evidence exists to verify the reactivity of acrolein with biological macromolecules.

Although there are no studies that provide adequate evidence for the carcinogenicity of acrolein following inhalation or oral exposure, evidence that glycidaldehyde, an acrolein metabolite, was carcinogenic in female ICR/Ha mice following subcutaneous injection was provided by Van Duuren et al. (1966).

6.4. POTENTIAL INVOLVEMENT OF PULMONARY LEUKOCYTES IN THE DEVELOPMENT OF LUNG TUMORS.

Phagocytic leukocytes have been shown by numerous investigators to be toxic to tumor cells *in vivo*, and increasing evidence suggests that cells of the mononuclear phagocyte series in particular may be of pivotal importance in providing protection against malignancy *in situ*. This protective function may be due, at least in part, to their ability to produce a tumor necrosis factor (TNF) (Urban et al., 1986). Whether the tumor surveillance and tumoricidal activities of AM (Hengst et al., 1978; Sone et al., 1983; Sone, 1986; Kan-Mitchell et al., 1985) are compromised or otherwise modified when they are engorged with even relatively benign particles has not been experimentally evaluated. Some existing information indicates that these AM activities can be modified by environmental factors (Sone et al., 1983; Flick et al., 1985). Additionally, the possibility remains that diesel and other types of particles at high lung burdens result in decreases in NK cell functional activities in providing defense against tumor formation either by direct particle-cell interactions or by altering the ability of AM to influence NK cell-mediated host defense against metastatic tumor cells (Sone, 1986). Moreover, the tumoricidal function of cytotoxic T lymphocytes (Sone, 1986) may be directly or indirectly compromised by the presence of high lung burdens of particles in the lungs. These possibilities, accordingly, require further investigation.

In addition to the release of TNF, another mechanism by which phagocytes may provide protection against tumor cells is via the production of toxic reactive species of oxygen (Clark and Klebanoff, 1975). Yet, this same process may mechanistically be a two-edged sword. Under some conditions the production of oxygen species may afford protection against emerging tumor cells by killing the cells, while under other conditions the production of reactive oxygen products conceivably may actually contribute to the development of neoplastic cells. Potential macrophage and polymorphonuclear neutrophil (PMN) involvement in the development of lung tumors in laboratory rats administered high lung burdens of diesel particles (Mauderly et al., 1987), or the inhalation of particles that are generally considered to have low to no cytotoxic potential [e.g. titanium dioxide (Lee et al., 1986)], over a prolonged period of time may be related to the ability of the lung free

cells to produce reactive oxygen metabolites during phagocytic oxidative metabolism (Hatch et al., 1980). Whereas products of phagocytic oxidative metabolism, including superoxide anion, hydrogen peroxide, and hydroxyl radical can kill tumor cells (Klebanoff and Clark, 1978), and the reactive oxygen species can peroxidize lipids to produce cytotoxic metabolites such as malonyldialdehyde, some products of oxidative metabolism apparently can also interact with DNA and produce mutations. Along this line, Weitzman and Stossel (1981) found that human peripheral leukocytes were mutagenic in the Ames assay. This mutagenic activity was related to polymorphonuclear leukocytes and blood monocytes; blood lymphocytes alone were not mutagenic. These investigators speculated that the mutagenic activity of the phagocytes was due to their ability to produce reactive oxygen metabolites inasmuch as blood leukocytes from a patient with chronic granulomatous diseases, a disease in which neutrophils have a defect in the NADPH oxidase generating system (Klebanoff and Clark, 1978), were less effective in producing mutations than were normal leukocytes. Of related significance in terms of oxygen species being able to cause genetic disturbances, Phillips et al. (1984) demonstrated that the incubation of Chinese hamster ovary cells with xanthine plus xanthine oxidase (a system for enzymatically generating active oxygen species) resulted in genetic damage hallmarked by extensive chromosomal breakage and sister-chromatid exchange, and produced an increase in the frequency of thioguanidine-resistant cells (HGPRT test). Aside from interactions of oxygen species with DNA, increasing evidence also points to an important role of phagocyte-derived oxidants and/or oxidant products in the metabolic activation of procarcinogens to their ultimate carcinogenic form (Kensler et al., 1987).

Hatch and co-workers (1980) have demonstrated that interactions of guinea pig AM with a wide variety of particles including silica, metal oxide-coated fly ash, polymethyl-methacrylate beads, chrysotile asbestos, fugative dusts, polybead carboxylate microspheres, glass and latex beads, uncoated flyash, and fiberglass increase the production of reactive oxygen species. Similar findings have been reported by numerous investigators for human, rabbit, mouse, and guinea pig AM (Drath and Karnovsky, 1975; Allen and Loose, 1976; Beall et al., 1977; Lowrie and Aber, 1977; Miles et al., 1977; Rister and Baehner, 1977; Hoidal et al., 1978; Drath, 1985). As well, polymorphonuclear leukocytes (PMN) are also

known to increase production of superoxide radical ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\cdot$) in response to membrane-reactive agents and particles (Goldstein et al., 1975; Weiss et al., 1978; Root and Metcalf, 1977). Thus, it appears that phagocytes from a variety of species produce elevated levels of oxidant reactants in response to challenges such as phagocytic stimuli, with the physicochemical characteristics of a phagocytized particle being a major factor in determining the magnitude of the oxidant-producing response.

It is well recognized that the deposition of particles in the lung can result in the efflux of PMN from the vascular compartment into the alveolar space compartment in addition to expanding the AM population size. Following acute exposures, the outpouring of the PMN is transient and generally over in a few days (Adamson and Bowden, 1978, 1981; Bowden and Adamson, 1978; Lehnert et al., 1988). The recruitment of this cell type in response to particle deposition has been related to chemotactic factors derived from the activation of complement (Warheit et al., 1985), the necessary components of which presumably are sufficiently available in the lung's ELF, and chemotactic factors that are elaborated by AM by phagocytic stimuli (Adamson and Bowden, 1982). Strom (1984) has reported that PMN become abnormally abundant following chronic exposures to particulate diesel exhaust. In the study by Strom (1984), the numbers of PMN lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled exposure mass concentration. Strom (1984) also found that PMN in diesel-exposed lungs remained persistently elevated for at least 4 mo after cessation of exposure. The duration of time over which AM continue to produce chemotactic factors after they have phagocytized particles has not been well characterized. However, a potential mechanism for the appearance of PMN at times well after particles have been deposited in the lungs may be related to an ongoing release of previously phagocytized particles by alveolar phagocytes that engulfed them shortly after deposition. Evidence in support of this possibility has been obtained by Lehnert et al. (1989). In the previously described study in which rats were intratracheally instilled with 85 μg, 1.06 mg, or 3.6 mg of polystyrene particles, PMN in lavaged lung free cell populations during the alveolar clearance of the three lung burdens of particles were quantitated. PMN were not found to be abnormally abundant during the clearance of the

two lower lung burdens, but they did become progressively elevated in the lungs of the animals in which alveolar phase clearance was restored. Moreover, the particle burdens in the PMN became progressively greater over time. Such findings are consistent with an ongoing particle relapse process, given the relatively short lifespan of PMN. As previously indicated, lung tumors develop in the rat at lesser lung burdens of diesel exhaust particles than with a particle like TiO_2. PMN characteristically are abnormally increased in the lung by diesel exhaust exposure, but their presence in the lungs does not appear to be excessive following the pulmonary deposition of even high lung burdens of TiO_2 (Strom, 1984; Lee et al., 1986). Thus, the generation of reactive oxygen species by both AM and PMN should be considered as one potential factor of what probably is a multistep process that culminates in the development of lung tumors in response to chronic particulate diesel exhaust deposition.

Another characteristic of the AM and PMN that may contribute to the pathologic process leading to lung tumor development following particulate diesel exhaust deposition is that these phagocytes are known to release a variety of potentially destructive hydrolytic enzymes. This release process is known to occur simultaneously with the phagocytosis of particles (Sandusky et al., 1977). The essentially continual release of such enzymes during chronic particle deposition and phagocytosis in the lung may be detrimental to the alveolar epithelium, especially Type I pneumocytes. Evans et al. (1986) showed that injury to Type I cells is followed shortly thereafter by a proliferation of Type II pneumocytes. Type II cell hyperplasia is a generally common feature observed in the lungs of animals that have received high lung burdens of various types of particles, including simple polystyrene micropsheres (see Figure 6-4). Exaggerated proliferation as a repair or defensive response to diesel deposition may have the effect of amplifying the likelihood of neoplastic transformation in the presence of carcinogens beyond that which would normally occur with lower rates of proliferation, assuming an increase in the cell cycling of target cells and the probability of a neoplastic-associated genomic disturbance.

The proliferative response of Type II cells following the deposition of particulate diesel or other types of particles, however, has yet to be directly related to a Type I cell destruction by proteolytic enzymes released by lung phagocytes or to a direct action of

particles on the proliferation kinetics of the Type II cells. The production of reactive oxygen species or products therefrom could also be involved in the process. Whatever the stimulus, it remains possible that the lung's AM population may play a role aside from any responsibility for Type I pneumocyte damage. AM have the ability to release several other effector molecules or cytokines that can regulate numerous functions of other lung cells, including their rates of proliferation (Bitterman et al., 1983; Jordana et al., 1988; Denholm and Phan, 1989). Am-derived mediators that may stimulate Type II cell hyperplasia following particle deposition in the lung, however, remain to be identified, if in fact the AM play a regulatory role in the Type II cell proliferative response.

6.5. SUMMARY OF METABOLISM AND MECHANISM OF ACTION OF CARCINOGENIC COMPONENTS OF DIESEL EXHAUST

Several studies affirm the bioavailability from inhaled diesel exhaust particles of compounds such as B[a]P and 1-NP which are known to be carcinogenic or mutagenic. Furthermore, that xenobiotics can undergo biotransformation to reactive intermediates following their entry into the body via inhalation of diesel exhaust particles has been demonstrated for B[a]P, 1-NP, and some of the dinitropyrenes. It is generally accepted that the underlying mechanism of carcinogenesis involves the formation of covalent adducts with DNA resulting in the alteration of cellular genetic information. Several reports have provided data indicating the formation of such adducts following administration of these compounds. A complete understanding of the carcinogenic potential of these compounds following diesel exhaust exposure appears to be dependent upon a thorough knowledge of metabolism and kinetic parameters relative to adduct formation and the involvement of genetic repair processes.

The development of lung tumors in experimental laboratory animals following chronic exposures to particulate diesel exhaust occurs under conditions in which alveolar macrophage-mediated particle clearance from the lung is compromised. Tumors have also been found to develop with other types of particles when this clearance mechanism is diminished. Thus, reductions in the functional activity of the lung's alveolar macrophage

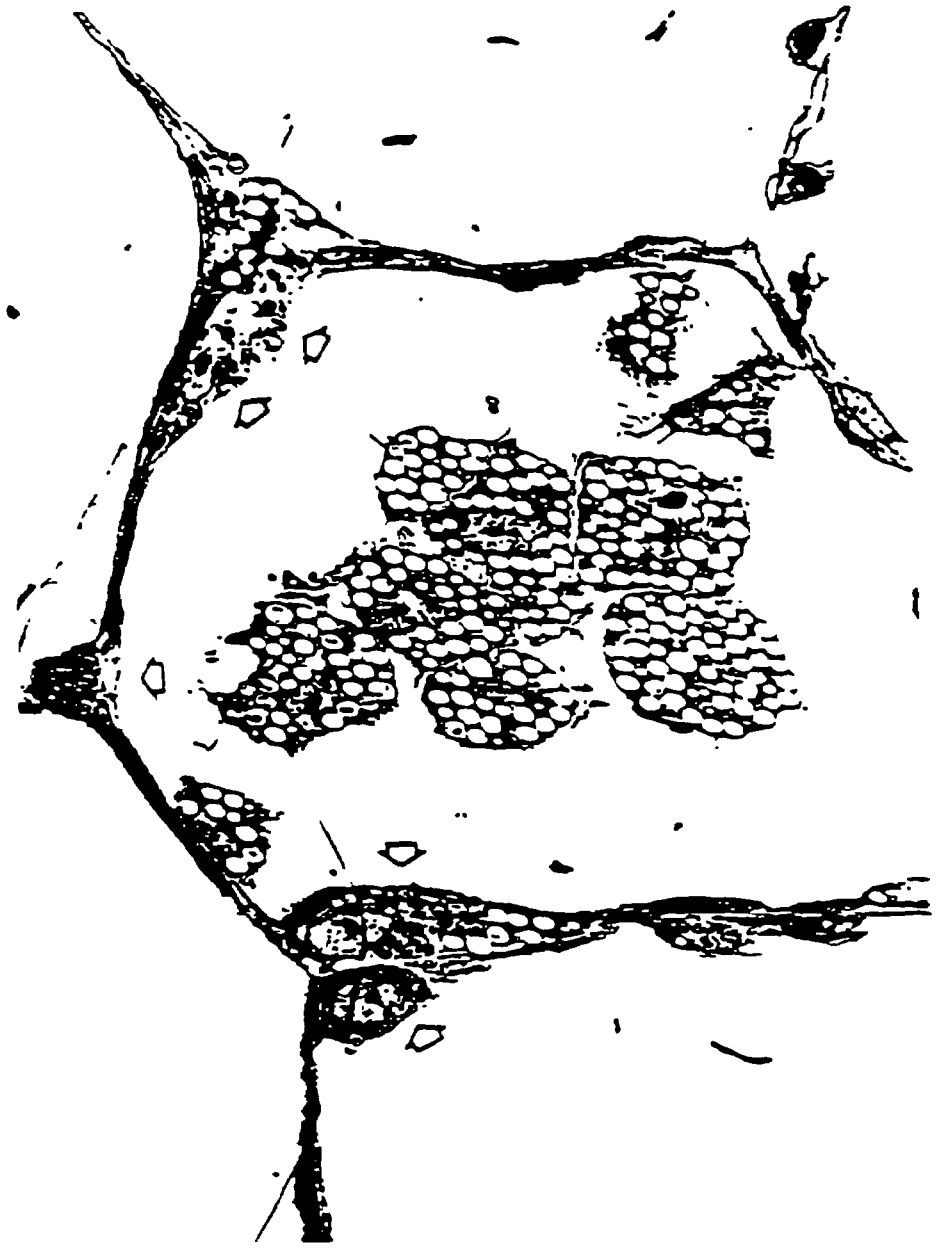

Figure 6-4. Electron micrograph of an aggregate of particle-filled AM in an alveolus on day 106 following the deposition of 6.8×10^8 microspheres. Arrows point to Type II pneumocytes which appear to be present in greater than normal abundance. The microspheres are also found in Type I epithelial cells.

population in the clearance process generally appear to be intimately related to the carcinogenic response to high lung burdens of particles. Findings that tumors develop in the lungs of laboratory rats at lesser lung mass or volume burdens of diesel particles than with a substance like titanium dioxide suggest that the carcinogenic response, however, is not exclusively related to an over abundance of particles in the lungs *per se*. Most likely, the organic components on diesel particles, many of which have demonstrated carcinogenic activity, are importantly involved in the development of lung tumors. The lung's pulmonary macrophages, which phagocytize deposited diesel particles, probably participate in the gradual *in situ* extraction and metabolism of procarcinogens associated with the diesel particles. Additionally, the normal tumoricidal activities of the pulmonary macrophages may be compromised upon interaction with excessive numbers of diesel particles. and diesel particle-macrophage interactions could lead to the generation of reactive oxygen species that have been shown to be at least mutagenic. Caution must be exercised in extrapolating observations made in animal models to the human condition. Processes and potential mechanisms discussed herein have largely been derived from animal data. Further research is required to determine how the activities of human pulmonary macrophages in response to particulate diesel exhaust compare with pulmonary macrophages from experimental animals. Most importantly, valid dosimetry for the human condition requires the elucidation of the underlying mechanisms involved in the development of lung tumors following chronic exposure to whole diesel exhaust.

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7. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

7.1 INTRODUCTION

Significant concern has been expressed regarding the potential carcinogenicity of diesel exhaust emissions, with special emphasis being placed on the particulate phase of the emissions. This particulate phase is composed of a carbon core representing about 80 percent of the total particle mass are adsorbed to this carbon core. A great variety of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), Benzo[a]pyrene and 1-nitropyrene are exhaust components that have received special attention regarding carcinogenicity. These organics may be strongly or weakly bound to the carbon core and represent varying amounts of the total particle mass. Qualitative and quantitative relationships for these organics depend on such variables as fuel quality, engine design, and engine operating conditions. The primary particle diameter ranges from 10 to 80 nm, but may form aggregates with mass median diameters of 0.2 to 0.3 μm (Vuk et al., 1976; Carpenter and Johnson, 1979). The ease with which these particles and their associated organics can be respired provides a basis for health hazard concerns. This fact and the reported mutagenicity (Huisingh et al., 1978) and carcinogenicity (Kotin et al., 1955) of solvent extracts of diesel soot have provided a rationale for examining the potential carcinogenicity of diesel emissions. Zamora et al. (1983) provided evidence that diesel exhaust particle extracts contained components that acted as weak tumor promoters *in vitro*. Recently, emphasis has been directed to assessing the carcinogenic potential of the particle-associated organics using whole animal studies and to understanding the mechanics and ramifications of deposition, retention, and clearance of the particle phase of diesel exhaust.

7.2 CARCINOGENICITY STUDIES IN LABORATORY ANIMALS

This chapter summarizes studies assessing the carcinogenic potential of diesel exhaust in laboratory animals. Experimental protocol for the inhalation studies consisted of exposure to diluted exhaust in whole animal exposure chambers (usually of chronic duration) using rats, mice, and hamsters as the model species. Some of these studies used both filtered (free of particulate matter) diesel exhaust and unfiltered (whole) diesel exhaust to differentiate gaseous phase effects from effects induced by the particulate matter and its adsorbed components. Diesel exhaust particulate matter concentrations ranged from 0.35 to 7.0 mg/m^3. Clean air was used in the control group exposures. Studies providing both positive and negative results as well as those indicating inconclusive findings have been reviewed.

Also included are studies that assessed the carcinogenic and/or tumorigenic effects of diesel exhaust particles and solvent extracts of these particles following dermal application, (s.c.) subcutaneous injection, (i.p.) intraperitoneal injection, or (itr.) intratracheal instillation in rodents.

7.2.1 Long-term Inhalation Studies

Mauderly et al. (1987) provided data affirming the carcinogenicity of automotive diesel engine exhaust on F344/Crl rats following chronic inhalation exposure. Male and female rats were exposed to diesel engine exhaust at nominal particulate matter concentrations of 0.35 (n = 366), 3.5 (n = 367), or 7.0 (n = 364) mg/m^3 for 7 h/d, 5 d/week up to 30 mo. Sham-exposed (n = 365) controls breathed filtered room air. The exhaust was generated by 1980 Model 5.7-L Oldsmobile V-8 engines operated through continuously repeating U.S. Federal Test Procedure (FTP) urban certification cycles. The engines were equipped with automatic transmissions connected to eddy-current dynamometers and flywheels simulating resistive and inertial loads of a mid-size passenger car. The D-2 diesel control fuel (Phillips Chemical Co.) met U.S. EPA certification standards and contained approximately 30 percent aromatic hydrocarbons and 0.3 percent sulfur. Following passage through a standard automotive muffler and tail pipe, the exhaust was diluted 10:1 with

filtered air in a dilution tunnel and serially diluted to the final concentrations. The primary dilution process was such that particle coagulation was retarded, and the serial dilution procedure was chosen because of a need for greater precision and stability of exhaust concentration. Flow through the 2.2-m^3 exposure chambers (H-200, Hazleton Systems, Aberdeen, MD) was set at 0.4 m^3/min, which provided an average residence time of 5.5 min in the chamber. Mokler et al. (1984) provides a detailed description of the exposure system. The gas-phase components of the diesel exhaust atmospheres are presented in Table 7-1. No exposure-related changes in body weight or life span were noted for any of the exposed

TABLE 7-1. GAS-PHASE COMPONENTS OF CONTROL AND DIESEL EXHAUST EXPOSURE ATMOSPHERES[a]

Component	Control	Low	Medium	High
Carbon monoxide (ppm)	1 (1)	3 (1)	17 (7)	30 (13)
Nitric oxide (ppm)	0	0.6 (0.3)	5.4 (1.5)	10.0 (2.6)
Nitrogen oxide (ppm)	0	0.1 (0.1)	0.3 (0.2)	0.7 (0.5)
Hydrocarbon vapor (ppm)	3 (1)	4 (1)	9 (5)	13 (8)
Carbon dioxide (percent)	0.2 (0.04)	0.2 (0.03)	0.4 (0.06)	0.7 (0.1)

[a]The mean (SE) of weekly mean values.

Source: Adapted from Mauderly et al., 1987.

animals, nor were there any signs of overt toxicity. Collective lung tumor incidence was greater (z statistic, $p \leq 0.05$) in the high (7.0-mg/m^3) and medium (3.5-mg/m^3) exposure groups (12.8 percent and 3.6 percent, respectively) vs. the controls and low (0.35-mg/m^3) exposure groups and (0.9 percent and 1.3 percent, respectively). Bronchoalveolar adenomas, adenocarcinomas, and squamous cysts (these were considered benign except for two that were classified as squamous cell carcinomas because of the presence of less differentiated cells and invasion of blood and lymph vessels) were identified. Using the same statistical

analysis on specific tumor types, adenocarcinoma plus squamous cell carcinoma and squamous cyst incidence was significantly greater in the high exposure group, and the incidence of adenomas was significantly greater in the medium exposure group. A significant ($p < 0.001$) dose-response relationship was obtained for tumor incidence relative to exposure concentration and lung burden of particulate matter (soot). These data are summarized in Table 7-2. A logistic regression model estimating tumor prevalence as a function of time, dose (lung burden of soot), and sex indicated a sharp increase in tumor prevalence for the high dose level at about 800 d after the commencement of exposure. A less pronounced, but definite increase in prevalence with time was predicted for medium and low dose levels. The model estimates closely agreed with the observed data. The particulate matter burden (mg/lung) of rats exposed to 0.35, 3.5, or 7.0 mg soot/m^3 for 24 mo was 0.6, 11.5, and 20.8, respectively, and served to affirm the greater accumulation that was due to decreased clearance following high exposure conditions. The greater incidence of lung DNA adducts for high-level exposed rats and the progressive accumulation of particulate matter in the lungs of these rats were used to support both genetic and epigenetic etiology of the observed lung tumors.

In summary, this study demonstrates a dose-dependent pulmonary carcinogenicity of diesel exhaust particulate matter following long-term inhalation exposure, and increased lung particulate matter burden resulting from this high-level exposure with subsequent decreased clearance. Furthermore, the logistic regression model indicated that both lung particulate matter burden and exposure concentration are useful as dose terms.

Wong et al. (1986) reported an increase in DNA adduct formation in male and female F344 rats exposed to whole diesel exhaust (7.1 mg particles/m^3) for 7 h/d (0800 - 1500 h), 5 d/week for 31 mo. The diesel exhaust was generated by a 5.7-L Oldsmobile engine burning U.S. EPA-certified fuel connected to an automatic transmission and an eddy-current dynamometer and was operated according to the (FTP) urban driving cycle (same emission generating system as described under Mauderly et al., 1987). The mass median diameter of the diesel exhaust particles was 0.23 μm. The gas-phase components of the diesel exhaust exposure and control atmospheres are listed in Table 7-3. Phosphorus-32 postlabeling was applied to DNA that was extracted from six control and six

TABLE 7-2. TUMOR AND LUNG PARTICULATE MATTER (SOOT) BURDEN IN MALE AND FEMALE F344 RATS EXPOSED TO DIESEL EXHAUST FOR 24 MO

Exposure concentration (mg/m^3)	Lung soot burden (mg/lung)	Percentages of rats with tumors			
		Adenomas	Adenocarcinomas + squamous cell carcinomas	Squamous cysts	All tumors
7.0 (high)	20.8±0.8	0.4	7.5*	4.9*	12.8*
3.5 (medium)	11.5±0.5	2.3*	0.5	0.9	3.6*
0.35 (low)	0.6±0.02	0	1.3	0	1.3
0 (control)	0	0	0.9	0	0.9

*Significant increase relative to control at $p < 0.05$, z-test, 364 to 367 rats per group.

Source: Adapted from Mauderly et al., 1987.

TABLE 7-3. GAS-PHASE COMPONENTS OF CONTROL (CLEAN AIR)AND DIESEL EXHAUST ATMOSPHERES

Components	Exposed rats		Control rats	
	X	SD	X	SD
Particles $\mu g/m^3$	7082	808	13	6
Carbon dioxide ppm	6643	1320	2005	290
Carbon monoxide ppm	29.7	12.9	1.0	0.7
Hydrocarbon vapors ppm	13.4	8.3	2.6	0.6
Nitrogen dioxide ppm	0.68	0.48	0[a]	0[a]
Nitrogen oxide ppm	10.0	2.6	0[a]	0[a]
Ammonia ppm	0.7	0.6	1.1	3.0

[a]Below detection limits.

Source: Wong et al., 1986.

exhaust-exposed rats (males and females). Characterization of the adducts and identification of the exhaust components responsible for their formation were not within the scope of the study. The lungs of exhaust-exposed rats were darkly pigmented and contained diesel particle-laden macrophages. Aggregates of these macrophages were frequently associated with alveolar wall fibrosis, bronchiolar metaplasia, and, occasionally, squamous metaplasia. Lungs from control rats were not darkly pigmented and had relatively unaltered airway and alveolar macrophage structure. Autoradiographic analysis revealed a higher intensity and elevated levels of DNA adducts in the exhaust-exposed rats. The authors indicated that quantitative and qualitative data regarding DNA adducts resulting from diesel exhaust exposure may be useful for extrapolation to potential effects in humans.

Lifetime inhalation exposure (19 h/d, 5 d/week) of female Wistar rats and male and female NMRI mice and Syrian golden hamsters to filtered and unfiltered (total) diesel

exhaust (particulate matter concentration of 4 mg/m^3) was studied by (Heinrich et al., 1986a,b). Animals were exposed for a maximum of 2.5 yr. The exposure system as described by Heinrich et al. (1986b), used a 40 kw 1.6-L diesel engine operated continuously under the U.S. 72 FTP driving cycle. The engines used European Reference Fuel with a sulfur content of 0.36 percent. Filtered exhaust was obtained by passing engine exhaust through a Luwa FP-65 HT 610 particle filter heated to 80°C and a secondary series of filters (Luwa FP-85, Luwa NS-30, and Drager CH 63302) at room temperature. The filtered and unfiltered exhausts were each diluted 1:17 with filtered air and passed through respective 12-m^3 exposure chambers. Mass median diameter of the diesel exhaust particulate matter was 0.35±0.10 μm. The gas-phase components of the diesel exhaust atmospheres are presented in Table 7-4.

Hamsters exposed to total or filtered diesel exhaust exhibited no significant difference in body weight gain relative to the control animals. Similarly, there was no significant change in body weight gain of rats or mice exposed to filtered exhaust. However, after approximately 480 d of exposure to total (unfiltered) diesel exhaust, rats and mice exhibited a decrease in weight gain and a weight loss, respectively, compared to the control animals and those exposed to filtered exhaust. For all three species, mortality (as determined by the median experimental lifetime) was not affected by either of the exposure protocols.

Lung weights (wet and dry) for all three species were significantly ($p \leq 0.05$) increased in the total exhaust exposure groups, but not in those animals exposed to filtered exhaust or clean air. Sex-dependent differences were not detected. Mechanical lung function parameters including tidal volume, minute volume, compliance, resistance, resistance with acetylcholine, functional residual capacity, and respiratory rate were also assessed. A significant ($p \leq 0.05$) reduction in airway resistance in hamsters was detected after 1 y of exposure, and significant reductions in resistance and compliance were noted for rats after 2 y exposure to total diesel exhaust. No significant changes in lung mechanics were reported for animals exposed to filtered exhaust or clean air. The lung mechanic tests were not performed using mice.

The total exhaust exposure protocol resulted in a 16-percent tumor incidence for female SPF Wistar rats. In addition to the bronchio-alveolar adenomas and squamous cell

TABLE 7-4. GAS-PHASE COMPONENTS OF CONTROL AND DIESEL EXHAUST ATMOSPHERES[a]

Component	Exhaust dilution (exhaust/exhaust + air) 1/17		
	Control (filtered air)	Filtered exhaust	Total exhaust
CO (ppm)	0.16±0.27	11.1±1.92	12.5±2.18
CO_2 percent	0.10±0.01	0.35±0.05	0.38±0.05
SO_2 (ppm)	--	1.02±0.62	1.12±0.89
NO_x (ppm)	--	9.9±1.80	11.4±2.09
NO (ppm)	--	8.7±1.84	10.0±2.09
NO_2 (ppm)	--	1.2±0.26	1.5±0.33
C_nH_m (ppm)	3.5±0.29	5.2±0.65	5.5±0.69
CH_4 (ppm)	2.3±0.17	2.4±0.20	2.6±0.19
C_nH_m without CH_4 (ppm)	1.3±0.15	2.9±0.50	3.1±0.53

[a]Mean±SD.

Source: Adapted from Heinrich et al., 1986b.

tumors, there was a high incidence of bronchio-alveolar hyperplasia (99 percent) and metaplasia of the bronchio-alveolar epithelium (65 percent). After 3 and 8 mo of exposure, these rats (n = 95) also exhibited a 2.5-fold increase in clearance halftime for a radioactive test aerosol ($^{59}Fe_2O_3$). This decreased clearance, however, was partially ameliorated after 12 and 19 mo. Filtered diesel exhaust was not tumorigenic to female Wistar rats (n = 92). Control rats (n = 96) exhibited no spontaneous tumor incidence. Following lifetime (19 h/d, 5 d/week, maximum of 120 weeks) exposure to filtered (n = 93) and unfiltered (n = 76) diesel exhaust, female NMRI mice exhibited a tumor incidence of 31 percent (12 percent adenomas, 19 percent adenocarcinomas) and 32 percent (15 percent adenomas, 17 percent adenocarcinomas), respectively (Heinrich et al., 1986b; Stober, 1986). Control mice (n = 84) had tumor incidences of 11 percent and 2.4 percent for adenomas and adenocarcinomas, respectively. When considering the incidence of only malignant tumors in the controls (2.4 percent), filtered exhaust (19 percent), and unfiltered exhaust (17 percent) groups, the effects of exhaust exposure are more dramatic. These data are summarized in Table 7-5.

No pulmonary tract tumors were observed for any of the 288 exhaust-exposed Syrian golden hamsters. Prior treatment with 4.5 mg diethylnitrosamine/kg, s.c., or 20 weekly intratracheal instillations of 250 μg B[a]P produced no significant evidence of syncarcinogenicity in hamsters exposed to diesel exhaust. Similar studies using mice (50 or 100 μg B[a]P intratracheally for 10 or 20 weeks, respectively, or 50 μg dibenz[a,h]anthracene intratracheally for 10 weeks) were inconclusive regarding the syncarcinogenicity of diesel exhaust. Rats pretreated with 25 weekly s.c. injections of 250 or 500 μg dipentylnitrosamine (DPN)/kg and exposed to unfiltered diesel exhaust exhibited a significantly increased incidence of squamous cell carcinomas of the lungs when compared to clean air controls. The rate of DPN-induced kidney and liver tumors was not influenced by exposure to total diesel exhaust. The study identified the rat as a sensitive model for diesel exhaust-induced tumorigenicity, but indicated that further investigations were required to ascertain the precise mechanism of tumorigenicity.

A long-term inhalation study (Ishinishi et al., 1986) examined the effects of emissions from light-duty (LD) and heavy-duty (HD) diesel engines on male and female Fisher 344/Jcl rats. The LD engines were 1.8-L, 4-cylinder, swirl chamber type power plants, and the HD

engines were 11-L, 6-cylinder, direct injection type power plants. The engines were connected to eddy-current dynamometers and operated at 1200 rpm (LD engines) and 1700 rpm (HD engines). Nippon Oil Co. JIS No. 1 or No. 2 diesel fuel was used. The 30-mo whole body exposure protocol (16 h/d, 6 d/week) employed diesel exhaust particle concentrations of 0, 0.4, 1, 2, or 4 mg/m^3 from HD engines and 0, 0.1, 0.4, 1, or 2 mg/m^3 from LD engines. Benzo(a)pyrene concentrations were reported as 4.4 and 2.8 μg/g of particulate matter, and 1-nitropyrene concentrations were 57.1 and 15.3 μg/g of particulate matter for the LD and HD engines, respectively. An analysis of gas-phase components are presented in Table 7-6. Inhalation chambers were maintained on a 12 h light-dark cycle at 23±2°C and 55 percent relative humidity. The animals inhaled the exhaust emissions from 1700 to 0900 h (although not specifically stated, it is assumed that this represents approximately 10 to 12 h of exposure during darkness). One hundred twenty male and 95 female rats were used for each exposure concentration.

The long-term exposure to diesel exhaust from LD and HD engines did not significantly affect the survival of the rats. Body weight data, presented for only female rats, indicated a 15- to 20-percent decrease in mean body weight relative to the controls. Indication of statistical significance was not provided for these data. Light microscopic examination revealed a dose- and time-dependent pulmonary burden of particles that was evident after 6 mo exposure to diesel emissions. Following 24 mo of exposure, the platelet count was significantly decreased in male rats of the 4-mg/m^3 exposure group. Significant decreases in serum cholinesterase, free cholesterol, and phospholipids were detected in rats exposed to 4 mg/m^3 of HD engine exhaust.

For the experiments using the LD series engines, the incidence of carcinomas was dose-independent with the highest incidence (4.1 percent) being in the 1-mg/m^3 exposure group. No significant difference was found among the exposure groups for the LD series engines. For the experiments employing the HD series engines the carcinoma incidence was dose-dependent with the highest incidence (6.5 percent) occurring in the 4-mg/m^3 exposure group. The only statistically significant difference reported was that between the 4-mg/m^3 and 0 mg/m^3 exposure groups for the HD series engines. Tumor incidence data for this

TABLE 7-6. GAS-PHASE COMPONENTS OF DIESEL EXPOSURE ATMOSPHERES

Experiment	Group mg/m^3	Gaseous concentration										
		NO_x ppm	NO ppm	NO_2 ppm	CO ppm	CO_2 %	SO_2 ppm	HTHC ppmc	LTHC ppmc	HCHO ppm	SO_4^{2} µg/m$_3$	O_2 %
Carcinogenicity & long-term inhalation (HD)	4	37 45	34 45	3 00	12 91	0 360	4 57	15 65	7.62	0.29	361	20.4
	2	21 67	19.99	1 68	7.75	0 215	2 82	9 94	5.90	0 18	198	20 6
	1	13 13	12.11	1.02	4 85	0 140	1.79	7.15	5.16	0 11	111	20.7
	0.4	6 17	5.71	0 46	2 65	0 084	0 98	4 63	4.27	0 05	62.9	20.8
	0	0 061	0.042	0 021	0.63	0.035	0 06	2 43	3.50	0.003	0.49	20.8
Carcinogenicity & long-term inhalation (LD)	2	18 93	20.34	1 41	7.10	0.418	4 70	5.49	3.57	0.13	315	20.3
	1	9 44	10.14	0 70	3.96	0 219	2.42	3 82	2.87	0.07	151	20.5
	0 4	3 81	4 06	0 26	2 12	0.105	1 06	2 93	2.51	0.03	62.4	20.7
	0.1	1 16	1.24	0 08	1.23	0.050	0.38	2 44	2.27	0.01	18.8	20 8
	0	0 033	0.044	0.011	0 80	0.026	0 06	2.20	2.17	0.002	0.41	20.8

Source: Adapted from Ishinishi et al , 1986

experiment are presented in Table 7-7. No information was reported regarding a correlation between carcinoma incidence and concentrations of benzo(a)pyrene and 1-nitropyrene.

Iwai et al. (1986) also examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 0.37-L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20 to 25 °C and 50 percent relative humidity in a 620-cm-long dilution tunnel with an inside diameter of 45 cm. The engines were operated at 1000 rpm with an 80 percent engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest NO_2 and SO_2 content. The exposure chambers were ventilated 10 times per hour and maintained at -5 to -7 mm H_2O. For those chambers using filtered exhaust, proximally installed HEPA filters were employed. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air 8 h/d, 7 d/week for 24 mo. Particle concentration was 4.9-mg/m^3 for unfiltered exhaust. Concentrations of gas-phase exhaust components were 30.9 ppm NO_x, 1.8 ppm NO_2, 13.1 ppm SO_2, and 7.0 ppm CO.

No lung tumors were found in the 2-yr control (filtered room air) rats, although one adenoma was noted in a 30-mo control rat providing a spontaneous tumor incidence of 4.5 percent. No lung tumors were observed for rats exposed to filtered diesel exhaust. Four of 14 rats exposed to unfiltered diesel exhaust for 2 yr developed lung tumors, two of these tumors being specified as malignant. Five rats of this 2-yr exposure group were subsequently placed in clean room air (3 to 6 mo) with four eventually (time not specified) exhibiting lung tumors (one malignancy). Thus, the lung tumor incidence for rats exposed to whole diesel exhaust was 42.1 percent (8/19) with 26.3 percent (5/19) being malignant. The tumors types identified were adenomas (3/19), adenocarcinomas (1/19), adenosquamous carcinoma (2/19), squamous carcinoma (1/19), and large-cell carcinoma (1/19). The lung tumor incidence in rats exposed to whole diesel exhaust was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-8. Malignant. splenic lymphomas were detected in 37.5 percent of the rats in the filtered exhaust group and in 25.0 percent of the rats in the unfiltered exhaust group, these values being significantly ($p \leq 0.05$) greater than the 8.2-percent incidence noted for the control rats. The study demonstrates production of lung cancer in rats following 2-yr exposure to unfiltered diesel exhaust. Additionally, the

TABLE 7-7. TUMOR INCIDENCE IN MALE AND FEMALE F344 RATS FOLLOWING LONG-TERM (30 MO) INHALATION EXPOSURE TO DIESEL EXHAUST

Exposure concentration mg/m³		Lung soot burden	Percentages of Rats With Tumors			
			Adenomas	Carcinomas[a]	All tumors	Total for group
LD						
2.0	M	NP	0 (0/64)	3.1 (2/64)	3.1 M	2.4
	F	NP	1.7 (1/60)	0 (0/60)	1.7 F	
1.0	M	NP	0 (0.64)	4.7 (3/64)	4.7 M	4.1
	F	NP	0 (0/59)	3.4 (2/59)	3.4 F	
0.4	M	NP	1.6 (1/64)	0 (0/64)	1.6 M	0.8
	F	NP	0 (0/61)	0 (0/61)	0 F	
0.1	M	NP	0 (0/64)	1.6 (1/64)	1.6 M	2.4
	F	NP	1.7 (1/59)	1.7 (1/59)	3.4 F	
Control						
	M	NP	0 (0/64)	3.1 (2/64)	3.1 M	3.3
	F	NP	1.7 (1/59)	1.7 (1/59)	3.4 F	
HD						
4.0	M	NP	0 (1/64)	7.8 (5/64)	7.8 M	6.5[b]
	F	NP	0 (0/60)	5.0 (3/60)	5.0 F	
2.0	M	NP	0 (0/64)	4.7 (3/64)	4.7 M	3.3
	F	NP	0 (0/59)	1.7 (1/59)	1.7 F	
1.0	M	NP	0 (0/64)	0 (0/64)	0 M	0
	F	NP	0 (0/64)	0 (0/64)	0 F	
0.4	M	NP	0 (0/64)	1.6 (1/64)	1.6 M	0.8
	F	NP	0 (0/59)	0 (0/59)	0 F	
Control						
	M	NP	0 (0/64)	0 (0/64)	0 M	0.8
	F	NP	0 (0/59)	1.7 (1/59)	1.7 F	

[a]Squamous cell carcinomas, adenosquamous carcinomas, adenocarcinomas, differentiation not provided
[b]Significant at $p < 0.05$ relative to controls
LD Light-duty engine
HD Heavy-duty engine
NP Not provided

Source: Adapted from Ishinishi et al., 1986

TABLE 7-8. TUMOR INCIDENCE IN FEMALE F344 RATS FOLLOWING LONG-TERM (24 MO) EXPOSURE TO FILTERED OR UNFILTERED DIESEL EXHAUST, OR CLEAN AIR

Exposure concentration (mg/m^3)	Lung soot burden	Percentage of rats with tumors						
		Adenomas	Adenocarcinomas	Adenosquamous carcinomas	Squamous carcinomas	Large cell carcinomas	Splenic lymphomas	All tumors[a]
4.9	NR	15.8 (3/19)	5.3 (1/19)	10.5 (2/19)	5.3 (1/19)	5.3 (1/19)	25 (6/24)	42.1 (8/19)[b]
Filtered	—	0	0	0	0	0	37.5 (9/24)	0
Clean air	—	0[c]	0	0	0	0	8.3 (2/24)	0

[a]Lung tumors only.
[b]Significant relative to controls at $p < 0.01$ (with Yates correction); 26.3 percent (5/19) malignancies, significant relative to control at $p < 0.05$ (with Yates correction).
[c]One microscopic adenoma found in 30-mo control rat during the last 6-mo observation period.

Source: Adapted from Iwai et al., 1986.

occurrence of splenic, malignant lymphomas was attributed to exposure to filtered and unfiltered diesel exhaust. It was not possible to ascertain if the splenic malignancies were due to the particulate phase of the diesel exhaust or to adsorbed organic compounds. It is possible that the gas phase of the exhaust was responsible for these lymphomas because they were detected in filtered exhaust. Other long-term studies apparently did not examine splenic tissue or did not detect such lesions.

A chronic (to 28 mo) inhalation exposure study by Takemoto et al. (1986) was conducted to determine the effects of diesel exhaust, di-isopropanol-nitrosamine (DIPN), and diesel exhaust following DIPN treatment on female F344 rats. Male and female C57Bl/6N mice and ICR mice were also used to determine the effects of diesel exhaust but were not treated with DIPN. DIPN was administered i.p. at 1 mg/kg weekly for 3 weeks to two groups of rats. A control group received neither treatment. Treatment protocol consisted of exposure to diesel exhaust for 4 h/d, 4 d/week. The diesel exhaust was generated by a 269-cc displacement engine operated at an idle state (1600 rpm). The exhaust was directed into a 104-L stainless steel storage chamber and diluted 1: to 1:4 in a 92.8-L PVC mixing chamber. The diluted exhaust then entered the inhalation chamber (376 L) at a flow rate of 120 L/min Pressure in the chamber was maintained at 5 mmAq. Gas-phase components of the exhaust were 40 to 100 ppm CO, 3 to 6 ppm NO, 2 to 4 ppm NO_2, and <0.5 ppm SO_2. Diesel exhaust particle concentration in the exposure chamber was 2 to 4 mg/m^3. The mean diameter of the particles was 0.32 μm. B[a]P and 1-nitropyrene concentrations were 0.85 and 93 μg/g particle, respectively.

After 1 week of exposure to diesel exhaust, deposition of black particles in alveolar spaces and bronchioles of the exposed animals was noted. At 1 mo of exposure the particle deposition extended to the alveolar walls and the peritracheal lymph nodes, and by 3 mo deposition beneath the pleura was observed. Further exposure resulted in greater particle deposition, but no quantitative data were provided. Rats exhibited a uniform particle deposition in all lobes of the lung, and mice exhibited regional accumulations of particles. Pneumonia, bronchitis, alveolar hyperplasia, and pituitary tumors were observed in rats of all treatment groups, but not in the controls, however, no quantitative data were provided relative to these findings.

No significant evidence of carcinoma formation was noted in any of the rats exposed to only diesel exhaust. One adenocarcinoma was observed in a control ICR mouse at 24 mo and a slight, but insignificant increase in adenocarcinomas (2/11 males, 1/11 females) was noted in diesel exhaust-exposed mice at 18 to 24 mo. For C57Bl mice, adenomas were observed in 2/40 males and 2/39 females at 13 to 18 mo and in 4/33 males and 4/38 females at 19 to 24 mo. Adenocarcinomas were noted for 2/33 males and 1/38 females at 19 to 24 mo. This was in contrast to one adenocarcinoma in a male control mouse. A slight but insignificant increase in tumor incidence was observed for both strains of mice exposed to diesel exhaust. The authors suggested, however, that mouse strains known for low spontaneous lung tumor occurrence may be less sensitive and thus be less likely to respond to tumorigenic agents. Tumor incidence data for the various treatment protocols are presented in Table 7-9. It was also noted that the small diesel engine used in this study had different operating characteristics than that of a diesel-powered automobile. A long-term inhalation study using female rats was conducted by Mohr et al. (1986) in which the effects of inhalation exposure to unfiltered diesel exhaust and PAH-enriched coal oven flue gas were compared. The diesel exhaust generating and exposure systems were those used by Heinrich et al., 1986a; 1986b). Briefly, exhaust was produced by a 40-kW, 1.6-L diesel engine operated continuously according to the U.S. 72 (FTP) test driving cycle and using European Reference Fuel with a 0.36 percent sulfur content. The exhaust was diluted 1:17 and delivered to the exposure chambers at a particle concentration of 3.9 mg/m^3. The mass median diameter of the diesel exhaust particles was 0.35 ± 0.10 μm. The dilution and filtering system are described fully in the Heinrich et al., 1986a study descriptions. An analysis of the gas-phase components of the diesel exhaust was not provided but assumed to be similar or identical to that reported by Heinrich et al. (1986a,b). Groups of 96 female Wistar rats were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or clean air for 18 h/d, 5 d/week for 2.5 yr.

Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to the unfiltered diesel exhaust. Squamous metaplasia was observed in 65.3 percent of the rats breathing unfiltered diesel exhaust but not in any of the control rats. Bronchio-alveolar neoplasms and squamous cell tumors were noted in 8 of 15 and 9 of 15 diesel

TABLE 7-9. TUMOR INCIDENCE IN ICR AND C57B6 MICE FOLLOWING LONG-TERM (24 MO) INHALATION EXPOSURE TO DIESEL EXHAUST AND IN RATS EXPOSED TO DIESEL EXHAUST WITH OR WITHOUT DIPN TREATMENT

		Percentage of animals with tumors								
Exposure group		Ad	Ad-Ca	Ca	Ad	Ad-Ca	Ca	Ad	Ad-Ca	Ca
F 344 Rats		Percent	6 mo	Percent	Percent	12 to 17 mo	Percent	Percent	18 to 24 mo	Percent
Diesel (2-4 mg/m^3)		0 (0/4)	–	0 (0/4)	0 (0/7)	–	0 (0/7)	0 (0/15)	–	0 (0/15)
DIPN		0 (0/3)	–	0 (0/3)	25 (2/8)	–	0 (0/8)	48 (10/21)	–	19 (4/21)
Diesel + DIPN		0 (0/5)	–	0 (0/5)	67 (12/18)	–	17 (3/18)	67 (12/18)	–	39 (7/18)
Control		0 (0/3)	–	0 (0/3)	0 (0/3)	–	0 (0/3)	0 (0/3)	–	0 (0/3)
ICR Mice		7-12 mo			13-18 mo			19-28 mo		
Diesel (2-4 mg/m^3)	M	0 (0/29)	0 (0/29)	–	13 (2/15)	7 (1/15)	–	27 (3/11)	18 (2/11)	–
	F	0 (0/20)	0 (0/20)	–	11 (2/19)	0 (0/19)	–	27 (3/11)	9 (1/11)	–
Control	M	0 (0/19)	0 (0/19)	–	11 (2/19)	0 (0/19)	–	10 (1/10)	0 (0/10)	–
	F	0 (0/17)	0 (0/12)	–	5 (1/19)	0 (0/19)	–	17 (2/12)	8 (1/12)	–
C57B6										
Diesel (2-4 mg/m^3)	M	0 (0/28)	0 (0/28)	–	5 (2/40)	3 (1/40)	–	12 (4/33)	6 (2/33)	–
	F	0 (0/10)	0 (0/10)	–	5 (2/39)	3 (1/39)	–	11 (4/38)	3 (1/38)	–
Control	M	0 (0/14)	0 (0/4)	–	0 (0/7)	0 (0/7)	–	6 (1/17)	0 (0/17)	–
	F	0 (0/14)	0 (0/4)	–	0 (0/12)	0 (0/12)	–	0 (0/15)	0 (0/15)	–

Ad: Adenoma.
Ad-Ca: Adenocarcinoma.
Ca: Carcinoma.
DIPN. di-isopropanol nitrosamine.

Source adapted from Takemoto et al., 1986.

exhaust-treated rats, respectively. One of the nine squamous cell tumors was characterized as a Grade I carcinoma, while the remaining eight were classified as benign, keratinizing, cystic tumors. Although both the diesel exhaust-exposed and the coal oven flue gas-exposed rats exhibited a greater incidence of neoplastic and non-neoplastic lesions compared to the control animals (Tables 7-5 and 7-10), statistical analysis was not provided for these data. Rats exposed to the diesel exhaust had a greater incidence of non-neoplastic lesions than did the flue gas-exposed rats with both exhibiting greater incidence than control rats, but again statistical evaluation of the datawas not provided.

For comparison, rats were also exposed to coal oven flue gas mixed with pitch fumes that had been pyrolized under nitrogen. Of 120 rats exposed to PAH-enriched coal oven flue gas, there were 16 benign, keratinizing, cystic tumors and 2 Grade-II carcinomas. The concomitance of severe inflammatory changes such as hyperplasia and metaplasia development of squamous tumors in the lungs of diesel exhaust-exposed rats, and the absence of such a relationship in rats exposed to PAH-enriched coal oven flue gas, provided the basis for the authors' contention that diesel exhaust may have more of a promotional effect than a complete carcinogen effect. However, diesel fumes may have direct-acting carcinogenic properties in addition to those inducing lung pathology so this study does not offer proof of Heinrichs theory. This study, apparently a phase of the research reported by Heinrich et al. (1986a,b), provides more detailed histological interpretation of the lesions produced following inhalation exposure to diesel exhaust and PAH-enriched coal oven flue gas. Additionally, it provides evidence for induction of neoplastic lesions following long-term inhalation exposure to unfiltered diesel exhaust, but does not provide information linking the tumorigenic effects to either the particulates or adsorbed organic compounds.

Brightwell et al. (1986) studied the effects of gasoline engine exhaust and of filtered and unfiltered diesel exhaust on male and female Syrian golden hamsters and F344 rats. The hamsters of each treatment group were also pretreated with a single administration of 4.5 mg diethylnitrosamine (DEN) 3 d prior to exhaust exposure. The diesel exhaust was generated by a 1.5-L (manufacturer not provided) engine, computer-operated according to the U.S. 72 (FTP) driving cycle, and the gasoline engine exhaust was produced by a 1.6-L engine. The engine emissions were diluted by conditioned air delivered at 800 m^3/h to

TABLE 7-10. PULMONARY LESION INCIDENCE IN FEMALE WISTAR RATS FOLLOWING LONG-TERM (24 to 30 MO) INHALATION EXPOSURE TO UNFILTERED DIESEL EXHAUST

Treatment	Percentages of animals with non-neoplast lesions		
	Bronchio-alveolar hyperplasia	Septal thickening	Squamous metaplasia
Diesel (3.9 mg/m³) exhaust	98 9 (94/95)	97.9 (93/95)	65.3 (62/95)
	43.1 (50/116)	26.7 (31/116)	30.2 (35/116)
	1.0 (1/96)	8 4 (8/96)	0 (0/96)

Source: Adapted from Mohr et al , 1986.

produce the high exposure (6.6 mg/m^3, diesel exhaust) atmosphere. Further dilutions of 1:3 and 1:9 produced the medium (2.2 mg/m^3, diesel) and low (0.7 mg/m^3, diesel) exposure atmospheres. Filtered diesel exhaust was generated by a similar engine equipped with a particle filter. The CO and NO_x concentrations were 32±11 and 8±1 for the unfiltered diesel exhaust (high exposure concentration chamber) and 32±11 and 8±1 for the filtered diesel exhaust. CO and NO_x concentrations for the gasoline engine exhaust were 224±32 and 49±5 ppm, respectively. Passage of this exhaust through a catalytic converter reduced these values to 21±10 and 7±3 ppm, respectively. The inhalation exposures were conducted overnight with five 16-h periods per week for two yr.

The body weights of rats were significantly lower (*p* value and statistical test not indicated although t-tests were used for analysis of other parameters) in both medium and high diesel exhaust exposure groups. These weight changes were evident shortly after the beginning of exposure and became more significant with increasing exposure duration. The raw data were not presented for these findings, nor were there any comments regarding body weights of the hamsters. None of the exposure treatments resulted in significant changes in urinalysis parameters. Blood chemistry analyses for rats indicated significant (*p* values ranging from 0.05 to 0.001, t-test) elevations of blood urea nitrogen, alkaline phosphatase, and aspartate aminotransferase in male rats and significant increases in blood urea nitrogen and alkaline phosphatase in female rats. Significant decreases in glucose, total proteins, triglycerides, and cholesterol were reported for female rats exposed to diesel exhaust for 18 to 24 mo. For male rats, decreases were noted for glucose, cholesterol, and cholinesterase. These blood chemistry values were for 18- and 24-mo high exposure animals only. Blood chemistry values for hamsters following 16 mo exposure to the high concentration diesel exhaust indicated significant elevations in albumin and gamma-glutamyl transpeptidase in female hamsters, but only gamma-glutamyl transpeptidase was elevated in males. Significant decreases in potassium, lactate dehydrogenase, alpha-hydroxybutyric dehydrogenase, and aspartate aminotransferase were detected in female but not in male hamsters. The high exposure concentration of both diesel engine and gasoline engine exhaust elevated red blood cell counts, hemoglobin content, and hematocrit values in the rats. However, the diesel exhaust also caused an increase in white blood cells and

segmented neutrophils, and a decrease in lymphocytes in the rats. Only hemoglobin and hematocrit values were significantly increased in hamsters (males only) exposed for 16 mo to the high concentration of diesel exhaust.

A statistically significant increase in lung weight was noted for rats and hamsters exposed to diesel exhaust, but was more pronounced and progressive with exposure level and duration in the rats. The greatest increase in lung weight was observed in high exposure male rats at 30 mo (includes a 6-mo observation period) and the high exposure female rats at 24 mo at which times the lung weights were, respectively, 2.8-fold and 3.9-fold greater than the controls. Exposure of rats to the high level of diesel exhaust resulted in statistically significant (p values not provided), dose-related obstructive and restrictive airway effects. No further details of these pulmonary effects were provided in the cited report.

The cardiovascular system was also affected by the exhaust exposures. The heart-to-body weight ratio was significantly ($p < 0.001$) increased in rats exposed to gasoline engine exhaust. Left ventricular contractility was significantly ($p < 0.05$) lower, and right ventricular-heart weight ratio was significantly ($p < 0.001$) greater in diesel exhaust - exposed rats when compared to controls. Right ventricular weight-body weight ratio and total heart weight-body weight ratio were significantly ($p < 0.001$ and 0.01, respectively) greater in rats exposed to diesel exhaust than in controls.

Tumor incidence data for this study are presented in Table 7-11. A 4-percent (3/72) and 23-percent (16/71) incidence of primary lung tumors occurred in male F344 rats following exposure to 2.2 and 6.6 mg diesel soot/m^3, respectively. The tumor incidence in female rats was 15 percent (11/72) for the 2.2-mg/m^3 exposure group and 54 percent (39/72) for the 6.6-mg/m^3 exposure group. No significant increase was reported for male (1 percent, 1/72) or female (0 percent, 0/72) rats in the low exposure (0.7 mg/m^3) group compared with the control animals (male: 2 percent. 3/140: female: 1 percent. 1/142). Diesel exhaust-induced tumor incidence in rats was dose-dependent and higher in females than males. Similar to other studies, tumor incidence in rats was related to exposure to unfiltered as opposed to filtered exhaust. It must be noted, however, that the exposure during darkness (when increased activity would result in greater respiratory exchange and greater dose) could account, in part, for the high response reported for the rats. A detailed histological analysis

TABLE 7-11. TUMOR INCIDENCE IN MALE AND FEMALE F344 RATS AND SYRIAN GOLDEN HAMSTERS FOLLOWING LONG-TERM INHALATION EXPOSURE TO FILTERED OR UNFILTERED DIESEL EXHAUST WITH OR WITHOUT DEN[a] PRETREATMENT

Treatment		Lung soot burden	Percentages of animals with tumors[b]
Rats			
6.6 mg m^3	M	NR	23 (16/72)
	F	NR	54 (39/72)
2.2 mg/m^3	M	NR	4 (3/72)
	F	NR	15 (11/72)
0.7 mg/m^3	M	NR	1 (1/72)
	F	NR	0 (0/72)
Control	M	—	2 (3/40)
	F	—	1 (1/42)
Hamsters			
6 6 mg/m^3		NR	Respiratory tract tumors rare in non-DEN pretreated hamsters and were not related to exhaust exposure (t-test). Increased incidence of tracheal papillomas in all DEN-pretreated groups, but incidence was not related to exhaust exposures.
6 6 mg/m^3		NR	
2.2 mg/m^3		NR	
2.2 mg/m^3 DEN		NR	
0.7 mg/m^3		NR	
0.7 mg/m^3 DEN		NR	
Control		—	
Control + DEN		—	

[a]DEN: dimethylnitrosamine 4.5 mg/kg, s c., 3 d prior to exposure
[b]Primary lung tumors; specific histopathology not reported
NR: not reported

Source: Adapted from Brightwell et al , 1986

of tumor types was not provided. Although DEN-pretreated hamsters exhibited an increase in tracheal papillomas in all treatment and control groups when compared to non-DEN pretreated hamsters, there was no statistically significant (t-test) relationship between tumor incidence and exhaust exposure.

Karagianes et al. (1981) exposed male Wistar rats (40 per group) to diesel engine exhaust (8.3±2.0 mg soot/m^3), room air, diesel engine exhaust plus low-concentration coal dust, low-concentration coal dust only, or high-concentration coal dust 6 h/d, 5 d/week for up to 20 mo. The exhaust source was a 3-cylinder, 43-hp diesel engine using ASTM D-975 grade No. 2-D diesel fuel oil and Federal Specification VV-F-800 A grade DF-2 diesel fuel oil. This type of engine is normally used in mining situations and, as such, was connected to an electric generator and operated at varying loads and speeds to simulate operating conditions in an occupational situation. Controlling the CO concentration at 50 ppm, the exhaust was diluted 35:1 with compressed air. The exposure chambers were 3000-L plexiglass spheres of with diameters of 178 cm. The exposure chamber temperatures were 25 to 26.6 °C, relative humidity was 45 to 80 percent, and total flow through the chambers was 50 L/min. The mass median diameter of the diesel exhaust particles was 0.71 μm. The NO_2 concentration ranged from 4 to 6 ppm, the CO level was maintained at 50±3 ppm, and the SO_2 and aliphatic aldehyde levels remained below detection limits (1 ppm).

Exposure to diesel exhaust or coal dust alone, or in combination, failed to cause significant alteration of body weight or mortality in the rats. Blood samples of rats sacrificed at 4, 8, or 16 mo of exposure revealed no significant changes in hematocrit, erythrocyte, or differential leucocyte counts or in blood protein profiles. At 4, 8, 16 and 20 mo, a 4- to 5-percent increase in carboxyhemoglobin levels was noted in rats exposed to diesel exhaust or diesel exhaust plus coal dust. Histological examination of rats that died during the study and for those sacrificed at 4, 8, 16, or 20 mo of exposure revealed interstitial fibrosis, alveolar histiocytosis, and alveolar emphysema, the severity of which was related to exposure duration. There was no statistically significant ($p < 0.05$) difference in severity of lesions among the various exposure groups. One bronchiolar adenoma each was found in the diesel exhaust and diesel exhaust plus coal dust exposure groups; however, the occurrence was not statistically significant. Particle accumulation occurred at the terminal bronchioles,

respiratory bronchioles, alveolar ducts, and within alveolar sacs. Alveolar macrophages were heavily burdened with particles. No malignant respiratory tract tumors were found in any of the rats in any of the exposure groups, but soot samples taken from the diesel exhaust chamber and other system locations were mutagenic in the Ames assay (no specific data presented).

Male cynomolgus monkeys (15) and male (151) and female (72) F344 rats were exposed to diesel exhaust (2 mg/m^3) for 7 h/d, 5 d/week for 24 mo (Lewis et al., 1986). The same number of animals was also exposed to coal dust (2 mg/m^3), diesel exhaust plus coal dust (1 mg/m^3 for each component), or filtered air. The diesel exhaust was produced by a 7.0-L, four-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel (<0.5 percent sulfur by mass). The exhaust was passed through a Wagner water scrubber, which lowered the exhaust temperature and quenched engine backfire. The engine was connected to an Eaton-Dynamic Model 758-DG dynamometer and operated by a KIM-1 computer simulating the activity of a diesel-powered coal mining tram. The duty cycle included eight modes, of which idling constituted approximately 60 percent. The exhaust was diluted 27:1 prior to entering the exposure chambers. An analysis of the exposure atmospheres is presented in Table 7-12.

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of neoplastic lesions. It should be noted, however, that the 24-mo time frame employed in this study may not allow for prediction of tumors in primates. Tumors and premalignant conditions were not observed in the upper respiratory tract of treated rats, nor did the incidence of neoplasms in 50 tissues differ significantly from the control rats. Focal deposition of diesel exhaust particles in the lungs of both species was reported. Alveolar type II cell hyperplasia and lipidosis were demonstrated in the rats exposed to diesel exhaust. Mixed function oxidase activity of the lungs and liver was not altered by the exposure to diesel exhaust. Pulmonary maximum expiratory flow rate was significantly reduced in monkeys exposed to diesel exhaust for 12, 18, and 24 mo. Overt signs of toxicity were not demonstrated by body weight changes, organ/body weight ratios, or clinical chemistry indices.

Pepelko and Peirano (1983) summarized an extensive series of studies on the health effects of diesel emissions. The exposure system consisted of twenty-four 2.8-m^3 stainless

TABLE 7-12. ANALYSIS OF FRESH AIR CONTROL (FA), DIESEL EXHAUST (DE), COAL DUST (CD), AND COAL DUST PLUS DIESEL EXHAUST (DECD) ATMOSPHERES

Component	Unit	FA	CD	DE	DECD
Total particles	mg/m^3	a	4.98±0.82[b]	a	3.23±0.60
Respirable particles	mg/m^3	a	2.00±0.41	1.95±0.25	2.02±0.30
CO_2	percent	0.08±0.02	0.09±0.05	0.20±0.06	0.20±0.07
CO	ppm	2.2±0.9	2.2±0.9	1.5±3.1	10.9±2.8
NO	ppm	0.08±0.14	0.08±0.29	8.7±3.6	8.3±3.2
NO_2	ppm	0.06±0.04	0.06±0.05	1.5±0.5	1.6±0.5
THC (cold)[c]	ppm C	4.1±1.9	a	7.5±2.2	7.4±2.0
THC (hot)	ppm C	a	a	a	8.3±1.9
SO_2	ppm	a	0.01±0.07	0.81±0.38	0.61±0.29
NH_3	ppm	0.52±0.28	0.57±0.52	0.64±0.71	0.48±0.55
Total aliphatic aldehydes	ppm	0.02±0.01	0.02±0.01	0.12±0.06	0.12±0.05
SO_4^{2-}	ppb	a	16.8±17.9	29.0±24.9	42.3±33.8
$^{N}H_4$	ppb	a	6.5±14.3	27.0±30.7	16.5±23.3
Acrolein	ppb	3.0±3.3	6.2±4.7	60.2±24.5	57.8±20.5
Acetaldehyde	ppb	1.5±3.5	0.9±2.5	38.7±15.3	37.7±14.0

TABLE 7-12. (Continued)

Component	Unit	FA	CD	DE	DECD
Formaldehyde	µg/m³	7.6±3.5	7.4±4.1	38.3±23.0	37.4±26.6
Silica	µg/m³	ND[a]	21.9±10.4	ND	ND
Silicon	µg/m³	ND	22.1±10.3	ND	ND
Benzo(a)anthracene	µg/m³	a	3.2±2.2	19.6±9.9	11.2±5.2
Benzo(k)flouranthene	µg/m³	a	a	5.6±2.3	3.6±2.4
Benzo(a)pyrene	µg/m³	a	a	13.5±6.8	10.2±6.5
Flouranthene	µg/m³	a	26.5±11.5	139.3±98.1	67.5±52.4
Pyrene	µg/m³	a	32.3±15.1	123.4±72.2	60.0±36.6

[a]Not measured.
[b]Mean±SD.
[c]Total hydrocarbons.
ND = not detected; limits of detection for silica and silicon were 12 µg/m³ and 11 µg/m³, respectively.

Source: Adapted from Lewis et al., 1986.

steel exposure chambers. Exhaust was provided by two Nissan CN 6-33, 6-cylinder, 3.24-L diesel engines coupled to a Chrysler A-272 automatic transmission and Eaton model 758-DG dynamometer. The exhaust was mixed with filtered, conditioned air in a dilution tube and further mixed in a large-volume mixing chamber. The flow rate through the exposure chamber allowed for 15 changes per hour. Sixty-day pilot studies were conducted where a near-maximum tolerated exposure of diesel exhaust (1:14 dilution) provided 6-mg/m^3 particle concentrations. The engines were operated using the Modified California Cycle. These 20-h/d, 7-d/week exposures using rats, cats, guinea pigs and mice produced decreases in weight gain and food consumption. Therefore, the long-term studies reduced the exposure time to 8 h/d at an exhaust particle concentration of 6 mg/m^3 followed by another 12-mo period of exposure using a particle concentration of 12 mg/m^3. For these studies the engines were operated using the Federal Short Cycle. Analysis of the exhaust components are summarized in Table 7-13.

Some short-term studies (Orthoefer et al., 1981) also employed exposure of mice to irradiated exhaust (to simulate sunlight exposure). Groups of 25 male Strong A mice were exposed to irradiated or nonirradiated diesel exhaust for 20 h/d, 7 d/week for 7 weeks. The exhaust was produced by a 198-in^3 Nissan automotive diesel coupled to a Chrysler Torque-Flite transmission and absorption dynamometer. No. 2 diesel fuel was used by the engines. The engine was operated continuously for 20 h daily for the irradiated exhaust exposure and for 8 h daily for the nonirradiated exhaust exposures. Exhaust was diluted 1:13 with filtered air. The diluted exhaust then passed through a large-volume mixing chamber and into the exposure chambers; some of the exhaust from the mixing chamber was directed into a dynamic flow irradiation chamber to simulate sunlight.

The diesel exhaust exposures did not produce any significant signs of gross toxicity or affect the growth rates of the mice. Exposures to irradiated exhaust, nonirradiated exhaust, or control atmospheres were not significantly different relative to lung tumor incidence. The authors considered the results of the diesel exhaust exposure to be negative, although the following tumor incidence data were provided: control female mice (4/58); historical controls male mice (10/60); urethane-treated mice (9/52); diesel exposed (14/56); diesel plus urethane (22/59).

TABLE 7-13. CONCENTRATION OF GAS-PHASE COMPONENTS AND PARTICLES IN EXPOSURE CHAMBERS

Component	Units	Clean air chambers	Exhaust chambers	
		Weeks 1-124	Weeks 1-61	Weeks 62-124
CO_2	percent	0.04±0.002[a]	0.30±0.04	0.52±0.04
CO	ppm	2.20±0.50	20.17±3.01	33.30±2.94
Total Hydrocarbons (cold)	ppm	2.82±0.50	7.93±1.42	11.02±1.04
NO	ppm	0.05±0.04	11.64±2.34	19.49±3.80
NO_2	ppm	0.03±0.03	2.68±0.80	4.37±1.19
SO_2	ppm	0.03±0.02	2.12±0.58	5.03±1.03
Dilution factor	DF	—	18.16±1.72	9.37±1.13
Particulate mass	mg/m^3	0.00	6.34±0.81	11.70±0.99
Acrolein	ppm	—	0.025±0.003[a]	0.034±0.009
Formaldehyde	ppm	—	0.106±0.029	0.251±0.059
Total aliphatic aldehydes	ppm	—	0.177±0.043	0.338±0.057

[a]Standard deviation of weekly means.

Source: Adapted from Pepelko and Peirano, 1983

Small and nonsignificant increases in tumor incidence were found for male and female Strong A and Jackson A mice following 8-week inhalation exposure to whole exhaust or irradiated whole exhaust at a particle concentration of 6 mg/m^3 (Table 7-14) followed by holding in clean air until sacrifice at about 9 mo of age (Orthoefer et al., 1981 as reported in Pepelko and Peirano, 1983). Male and female Strain A mice were exposed to diesel exhaust (6 mg/m^3) from 8 weeks to 9 mo of age. Sixty animals each from the exposure and control groups also received a single i.p. dose of 1 mg urethane, a carcinogen initiator. Relative to respective clean air controls, females exposed to diesel exhaust or diesel exhaust and urethane pretreatment showed a slight but significant (p <0.01 for exhaust alone; p <0.02 for exhaust plus urethan pretreatment) increase in tumor incidence (Table 7-15). However, if these results are combined with the data for the much larger groups of male mice, which showed no increase in tumor incidence following any of the treatments, the controls and treatment groups are not significantly different.

Furthermore, it was noted that the tumor incidence (0.09 tumors per mouse) in the control group was less than the expected value of 0.25 tumors per mouse. Personal communication with the author indicated that in the case of the Strong A mice (1-mg urethane study), genetic drift had occurred in the main colony and the background tumor incidence and presumably sensitivity had decreased, therefore suggesting that the increased incidence in the female mice may, in fact, have been real.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust with and without pretreatment with tumor initiators. Exposure conditions were as described previously for Pepelko and Peirano (1983). Male and female mice were exposed to a diesel exhaust particle concentration of 6 mg/m^3 prior to and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) received an i.p. injection of butylated hydroxytoluene (BHT) (300 mg/kg for week 1, 83 mg/kg for week 2, and 150 mg/kg from week 3 to 1 year), a single i.p injection of 1 mg urethan, or no injections of tumor initiators. The exhaust exposure was increased to a particle concentration of 12 mg/m^3 when the offspring were 12 weeks of age and was maintained until termination of the experiment when the mice were 15 mo old. The incidence of pulmonary adenomas was significantly (p <0.02)

TABLE 7-14. LUNG TUMOR INCIDENCE IN STRAIN A MICE EXPOSED FOR 8 WEEKS TO RAW OR IRRADIATED EXHAUST AT A PARTICLE CONCENTRATION OF 6 mg/m^3

Treatment	Sex	Survivors/ initial	Mice With tumors	Tumors/ mouse
		STRONG A MICE		
Clean air	M	22/25	3[a]	0.13
Raw exhaust	M	19/25	7	0.63
Irradiated exhaust	M	22/25	6	0.27
		JACKSON A MICE		
Clean air	M	18/20	5	0.33
Clean air	F	18/20	11	0.66
Clean air	M & F	36/40	16	0.50
Raw exhaust	M	16/20	5	0.31
Raw exhaust	F	18/20	6	0 50
Raw exhaust	M & F	34/40	11	0.41

[a]The percentage of mice with tumors was compared using X^2 testing. No significant differences were detected among groups.

Source: Adapted from Pepelko and Pierano, 1983 tumor initiators.

TABLE 7-15. LUNG TUMOR INCIDENCE IN STRAIN A MICE EXPOSED TO DIESEL EXHAUST AT A PARTICLE CONCENTRATION OF 6 MG/M^3 UNTIL 9 MO OF AGE

Treatment	Sex	Survivors/ initial	Mice with tumors	p^a	Tumors/ mouse	p^a
Clean air	F	58/60	4		0.09	
Exhaust	F	56/60	14	<0.01	0.32	<0.01
Clean air + 1 mg urethan	F	52/60	9		0.25	
Exhaust + 1 mg urethan	F	59/60	22	<0.02	0.39	<0.01
Clean air	M	403/429	73		0.23	
Exhaust	M	368/430	66		0.20	

[a]X^2

Source: Adapted from Pepelko and Peirano, 1983

increased in the non-injected female mice exposed to diesel exhaust, but the exhaust exposure reduced ($p < 0.01$) the tumor incidence in female mice receiving BHT. Males were unaffected by either condition. All pulmonary tumors (adenomas and carcinomas) were significantly increased in female mice ($p < 0.05$) and male and females combined ($p < 0.05$). These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The lack of a clearly defined response may well have been influenced by the relatively early sacrifice times of the mice. These data are summarized in Table 7-16.

Additional studies in the Pepelko and Peirano report examined the effects on Strain A mice of diesel exhaust (particle concentration of 12 mg/m^3) following pretreatment with 5 mg urethan, the effects of exposure during night vs. daylight, and the effects of increasing exposure (12 mg particles/m^3) to 12 mo of age rather than 9 mo. These results are presented in Table 7-17. Tumor incidence was significantly reduced in the diesel exhaust/urethan treatment groups compared to animals receiving 5 mg urethan only. Exposure to diesel exhaust during the dark portion of the photoperiod or until 12 mo of age also reduced the tumor incidence when compared to control groups. The reasons for a decreased response to diesel exhaust exposure are unknown, although these greater exposure levels may have induced a greater degree of cell death in the cancerous than in normal cells.

General Motors Research Laboratories performed chronic inhalation studies using male F344 rats and Hartley guinea pigs exposed to diesel exhaust particle concentrations of 0.25, 0.75, or 1.5 mg/m^3 (Schreck et al., 1981). The exposure protocol was 20 h/d, 5.5 d/week for 24 mo. Exhaust was generated by a 5.7-L Oldsmobile engine (four different engines used throughout the experiment) operated at a steady speed and load simulating a 40 mph driving speed of a full-size passenger car. AMOCO Type 2D federal compliance fuel with a 0.27 percent sulfur content and AMOCO 200 30W oil were used. The mass median aerodynamic diameter of the exhaust particles was 0.2 μm with 90 percent of the mass associated with particles of less than 1.0 μm diam. Exhaust was directed into stainless steel lines and heated to 100±15 °C to simulate typical tail pipe temperatures. It was diluted with clean air and then entered the large volume (12.6 m^3) exposure chambers, which contained two cage racks

TABLE 7-16. EFFECTS OF INHALATION EXPOSURE TO DIESEL EXHAUST ON LUNG TUMOR INCIDENCE IN MALE AND FEMALE SENCAR MICE

Treatment group	Sex	Percent adenomas		Percent carcinomas		Percent all lung tumors	
		CA	DE	CA	DE	CA	DE
Uninjected	M	3.8	4.0	0.0	2.0	3.8	5.9
	F	6.3	16.3[a]	0.9	0.0	7.2	16.3[b]
	M & F	5.1	10.2[b]	0.5	1.0	5.6	11.2[b]
BHT	M	8.5	8.8	0.0	2.9	8.5	11.8
	F	16.7	3.9	1.5	2.6	18.1	6.5[b]
	M & F	12.2	5.4	1.7	2.7	12.8	8.1
Urethan	M	9.2	9.0	0.0	1.1	9.2	10.1
	F	7.0	8.4	1.8	3.7	8.7	12.1
	M & F	8.1	8.7	0.9	2.6	9.0	11.2
All treatments	M	7.1	6.7	0.0	1.7[a]	7.1	8.0
	F	8.9	10.1	1.4	2.1	10.3	12.2
	M & F	8.0	8.6	0.7	2.0	8.7	10.2

[a] $p = 0.02$
[b] $p \leq 0.05$

CA = Clean air.
DE = Diesel exhaust.
BHT = Butylated hydroxytoluene.

Source: Adapted from Pepelko and Peirano, 1983.

TABLE 7-17. LUNG TUMOR INCIDENCE IN STRAIN A MICE EXPOSED TO A PARTICLE CONCENTRATION OF 12 mg/m³

Treatment	Age (mo)	Illumination	Sex	Survivors/ initial	Mice with tumors	p^a	Tumors/mouse	p^b
Clean air	9	light	M	44/45	10		0 23	
			I	43/45	11		0 35	
			M & F	87/90	21		0 29	
Exhaust	9	light	M	37/45	5		0 19	
			F	43/45	4	<0 05	0 09	<0 05
			M & F	80/90	10	<0 05	0 14	<0 10
Clean air + 5 mg urethan	9	light	M	38/45	32		2 37	
			F	37/45	34		3 24	
			M & F	75/90	66		2 80	
Exhaust + 5 mg urethan	9	light	M	39/45	26	<0 10	1 03	<0 001
			F	36/45	16	<0 001	0 86	<0 0001
			M & F	75/90	42	<0 001	0 95	<0 0001
Clean air	9	dark	M	97/108	28		0 32	
			F	140/142	31		0 23	
			M & F	237/250	59		0 27	
Exhaust	9	dark	M	111/115	13	<0 01	0 14	<0 01
			F	139/143	9	<0 001	0 07	<0 001
			M & F	250/258	22	<0 001	0 10	<0 0001
Clean air	12	light	M	38/	22		0 68	<0 001
Exhaust	12	light	M	44/	11	<0 01	0 25	

[a]X^2
[b]Student's t-test.

Source: Adapted from Pepelko and Peirano, 1983

with up to 144 rats or 72 guinea pigs. Exposure chambers were maintained at 22±2°C at 50±20 percent relative humidity. Control animals received clean air.

Animals exposed to the diesel exhaust had discolored fur throughout the exposure period. Rats of the high exposure (1.5 mg/m^3) group exhibited body weights that were significantly less ($p < 0.05$, t-test) than that of controls. One group of guinea pigs exposed to the low (0.25 mg/m^3) exhaust particle concentration had body weights significantly lower ($p < 0.05$, t-test) than respective controls; however, this effect was not observed in animals of the higher exposure groups. No exposure-related overt signs of toxicity were reported for the test animals.

Ultrastructural changes in the lungs of the exposed guinea pigs were reported in Barnhart et al. (1981). No evidence was found for neoplastic changes, nor were pathological changes indicative of fibrosis or emphysema noted. After only 2 weeks of exposure and throughout the exposure period. significant uptake of exhaust particles by alveolar macrophages, epithelial type 1 cells. and interstitial macrophages was noted.

Takaki et al. (1989) exposed male and female F344 rats to whole exhaust from HD (11-L) and LD (1.8-L) engines using 13:15 and 10:15 dilution ratios, respectively. The rats (64 males and 59 females per group) were exposed to particle concentrations of 3.7, 1.8, 1.0 or 0.5 mg/m^3 (HD) or 0.1, 0.4, 1.1, or 2.3 mg/m^3(LD) for 16 h/d (1700-0900 h), 6 d/week for approximately 30 mo. Control groups were exposed to room air. Carbon particle deposition occurred primarily in the alveolar cavity for low particle concentration exposures. but was more prominent in the interstitium and lymphoid tissue at higher particle concentration exposures. Although the incidence of adenomatous hyperplasia increased with particle concentration for both the LD and HD groups, a significant difference in tumor incidence was noted only for the 3.7-mg/m^3 HD group (Table 7-18). For the LD groups, tumor incidence for the control, 0.1-. 0.4-. 1.1-. and 2.3-mg/m^3 exposure groups was 3.3. 2.4. 0.8. 4.1. and 2.4 percent, respectively. For the HD groups tumor incidence of 0.8, 0.8, 0.0, 3.3. and 6.5 was reported for the control, 0.5-, 1.0-, 1.8-, and 3.7-mg/m^3 groups, respectively.

TABLE 7-18. TUMOR INCIDENCE IN MALE AND FEMALE F344 RATS CHRONICALLY EXPOSED TO WHOLE EXHAUST FROM LD AND HD ENGINES

Treatment[a] (mg/m³)	Hyperplastic lesions	Adenomas	Carcinomas			Carcinoma incidence (%)	Total incidence[b] (%)
			Adeno	Adeno - squamous	Squamous		
LD							
0	4	1	0	2	1	2.4	8 (6.5)
0.1	4	1	1	0	1	1.6	7 (5.7)
0.4	6	1	0	0	0	0	7 (5.6)
1.1	12	0	5	0	0	4.1	17 (13.8)
2.3	87	1	1	1	0	1.6	90 (72.6)
HD							
0	1	0	1	0	0	0.8	2 (1.6)
0.5	3	0	0	0	1	0.8	4 (3.3)
1.0	7	0	0	0	0	0	7 (5.6)
1.8	14	0	3	1	0	3.3	18 (14.5)
3.7	25	0	5	1	2	6.5[c]	33 (26.6)

[a]n = 123 to 125 rats per group
[b]All tumors plus hyperplastic lesions.
[c]Significantly different from controls (p not specified)

Source: Adapted from Takaki et al., 1989.

7.2.2 Short-Term Inhalation and Intratracheal Instillation Studies

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that the carcinogenic potential of diesel exhaust resided in the polycyclic aromatic hydrocarbons (PAH) that consist of four or more rings. Condensate was obtained from the whole exhaust of a 3.0-L passenger car diesel engine connected to a dynamometer, the operation of which simulated city traffic driving conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25 percent and 75 percent of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (benzo[a]pyrene) controls were also included in the protocol (Table 7-19). Results of the various treatments are presented in Table 7-19. Fraction IIb (made up of PAHs with 4 to 7 rings), which accounted for only 0.8 percent of the total weight of diesel exhaust condensate, produced the highest incidence of lung carcinomas following implantation into the rat lungs. A carcinoma incidence of 17.1 percent was observed following implantation of 0.21 mg IIb/rat, whereas the nitro-PAH fraction (IId) at 0.18 mg/rat accounted for only a 2.8 percent carcinoma incidence. Hydrophilic fractions of the diesel exhaust particulate extracts, vehicle (beeswax/trioctanoin) controls, and untreated controls failed to exhibit carcinoma formation. Administration of all hydrophobic fractions (IIa-d) produced a carcinoma incidence (20 percent) similar to the summed incidence of fraction IIb (17.1 percent) and IId (2.8 percent). The benzo[a]pyrene positive controls (0.03, 0.1, 0.3 mg/rat) yielded a carcinoma incidence of 8.6 percent, 31.4 percent, and 77.1 percent, respectively. The study showed that the tumorigenic agents were primarily 4 to 7 ring PAHs and to a lesser extent nitroaromatics. It did not, however, provide any information regarding the bioavailability of the particle-associated PAHs assumed to be responsible for carcinogenicity.

Kunitake et al. (1986) reported an insignificant (2.3 percent) increase in tumor incidence in female Syrian hamsters ($n = 62$) to which a total dose of 7.5 mg of a methylene chloride extract of diesel exhaust tar (DET) had been instilled intratracheally over 15 weeks. Addition of 7.5 mg B[a]P to a total DET dose of 1.5 mg resulted in a tumor incidence of

TABLE 7-19. TUMOR INCIDENCE AND SURVIVAL TIME OF RATS TREATED WITH FRACTIONS FROM DIESEL EXHAUST CONDENSATE (35 RATS/GROUP)

Material portion by weight (%)	Dose (mg)	Median survival time in weeks (range)	No. of carcinomas[a]	No. of adenomas[b]	Carcinoma incidence (%)
Hydrophilic fraction(I) (25)	6 70	97(24-139)	0	1	0
Hydrophobic fraction (II) (75)	20.00	99(50-139)	5	0	14.2
nonaromatics +					
PAC 2 + 3 rings (IIa) (72)	19 22	103(25-140)	0	1	0
PAH 4 to 7 rings (IIb) (0.8)	0.21	102(50-140)	6	0	17.1
polar PAC (IIc) (1.1)	0.29	97(44-138)	0	0	0
nitro-PAH (IId) (0.7)	0.19	106(32-135)	1	0	2.8
Reconstituted hydrophobics (Ia), b, c, d) (74.5)	19.91	93(46-136)	7	1	20 0
Control, unrelated		110(23-138)	0	0	0
Control (beeswax/trioctanoin)		103(51-136)	0	1	0
Benzo[a]pyrene	0.3	69(41-135)	27	0	77 1
	0 1	98(22-134)	11	0	31.4
	0.03	97(32-135)	3	0	8.6

[a]Squamous cell carcinoma
[b]Bronchiolar/alveolar adenoma.

Source: Adapted from Grimmer et al., 1987.

91.2 percent with 88 percent of these being characterized as malignant. B[a]P (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2 percent (85 percent of these being malignant), which was not significantly different from the DET + B[a]P group. This subchronic study demonstrated a lack of detectable interaction between DET and B[a]P, the failure of DET to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of B[a]P. Intratracheal instillation of 0.03 μg B[a]P, the equivalent content in 15 mg DET, failed to cause a significant increase in tumor incidence in rats. Ishinishi et al. (1988) also reported this study. The effects on hamsters of intratracheally instilled whole diesel exhaust suspension, diesel particles with Fe_2O_3 as a carrier dust or diesel particle extract with Fe_2O as the carrier were studied by Shefner et al., 1982. The diesel exhaust component in each of the treatments was administered at concentrations of 1.25, 2.5, or 1.25 mg/week for 15 weeks to groups of 50 male Syrian Golden hamsters. The total volume instilled was 0.2 mL. The diesel particles and the dichloromethane extracts were suspended in physiological saline with gelatin (0.5 percent w/v), gum arabic (0.5 percent w/v), and propylene glycol (10 percent by volume). Fe_2O_3 concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles without the exhaust component. Two replicates of the experiments were performed.

At the time of the report, only preliminary results were available. A decrease in body weight gain was noted for those animals in the highest diesel exhaust particle and particle extract treatment groups. At the 12-mo sacrifice time (6 mo after the last treatment), alveolar macrophage phagocytosis of the lung particle burden was not yet complete. Adenomatous hyperplasia was reported as being most severe in those animals treated with the diesel exhaust particles or the diesel exhaust particles plus Fe_2O_3 particles, and least severe in those animals receiving the diesel particle extract suspension. Of the two lung adenomas detected microscopically, one was in a high-dose diesel particle-treated animal and the other was in a high-dose diesel extract suspension-treated animal.

Kaplan et al. (1982) conducted a 90-d inhalation exposure study using A/J mice. The animals were exposed to diluted diesel exhaust (1.5 ± 0.14 mg/m^3) or filtered air for 20 h/d, 7 d/week. As described by Schreck et al. (1981), the exhaust was produced by a 5.7-L

Oldsmobile engine operated continuously (steady-state mode) 20 h/d, 7 d/week at the equivalent of 40 mph. The engine exhaust passed into a stainless steel line which was heated to 100±15 °C to simulate tail pipe exhaust temperatures of motor vehicles and then rapidly diluted with clean air near the top of the exposure chambers. The stainless steel and glass exposure chambers (14.5 m^3) contained four cage racks each holding 14 cages. The temperature in the exposure chambers was 22±2 °C with a relative humidity of 50±10 percent. At the end of the exposure period, 30 control and 30 experimental animals were necropsied. Additional groups of control and experimental animals were used for analysis of pulmonary ultrastructure, morphometry, and proliferative changes. At 9 mo of age, 500 control and 500 diesel exhaust-exposed mice were used to study the pulmonary adenoma response. Mice injected with urethane (1 mg/kg, i.p.) at 2 mo of age and sacrificed four months later served as positive controls for pulmonary adenoma response. Six months after exposure, 30 control and 30 exposed mice were sacrificed for histopathology analysis.

No change in mortality, growth patterns, or pulmonary adenoma tumor incidence relative to control groups was observed. Tumor prevalence in the positive control A/J mice (1.0 mg urethane/kg, i.p.) was 100 percent. For diesel exhaust-exposed mice and controls, tumor incidence was 34.2 percent and 31.4 percent, respectively (Table 7-20).

The effect of chronic (2 to 2.5 yr) diesel exhaust exposure on the tumor-inducing effect of diphenylnitrosamine (DPN) was examined using female Wistar rats (Heinrich et al., 1989). Groups of rats (45 to 48 per group) were exposed to clean air, diluted (1:17) filtered diesel exhaust, or whole diesel exhaust (particle concentration of 4.24 mg/m^3) and administered 250 or 500 mg DPN/kg/week during the first 25 weeks of exposure. The total DPN dose was 6.25 or 12.5 g/kg of body weight. Exposure was for 19 h/d and 5 d/week. The exhaust was generated by engines (make not specified) that were operated using the U.S. 72 (FTP) driving cycle. The concentrations of B[a]P, B[e]P, and chrysene in the diesel exhaust were 13, 21, and 76 ng/m^3, respectively. The overall tumor rate in the lungs of DPN-treated rats was not affected by the exposure to either filtered or whole diesel engine exhaust. However, when only pulmonary squamous cell carcinomas were considered, the exposure to whole diesel exhaust significantly ($p \leq 0.05$) increased the tumor incidence (Table 7-21). Conversely, nasal tumor incidence was significantly decreased in the rats exposed to

TABLE 7-20. PULMONARY ADENOMA RESPONSE OF STRAIN A/J MICE EXPOSED TO DIESEL EXHAUST (1500 μg/m³ PARTICLES) FOR THREE MO

Treatment	Number of mice	Number of mice according to number of lung tumor/mouse							Total number	Mean number of lung tumors/mouse[a]	Percent prevalence
		0	1	2	3	4	13	>13			
Control	458	314	116	24	4	0	0	0	176	0.38±0.03	31.4
Exposed	485	319	133	26	5	1	1	0	217	0.45±0.04	34.2
Urethane (1 mg/g)	18	0	0	0	0	0	0	18	407	22.6±1.90	100

[a]Mean±S.E.

Source: Kaplan et al., 1982.

TABLE 7-21. TUMOR INCIDENCE IN FEMALE WISTAR RATS TREATED WITH DIPHENYLNITROSAMINE (DNP) AND EXPOSED TO CLEAN AIR (CA), FILTERED DIESEL EXHAUST (FDE), OR WHOLE DIESEL EXHAUST (DE)

Treatment	Tumor incidence (percent)		
	Squamous cell carcinomas (lung)	All tumors (lung)	All tumors (nasal cavity)
6.25 DPN/kg			
CA	4.4	84.8	28.3
FDE	4.4	67.4	4.4*
DE	46.8*	83.0	8.7*
12.5g DPN/kg			
CA	16.7	93.8	52.1
FDE	14.6	89.6	31.3*
DE	31.3*	89.6	22.9*

*$p \leq 0.05$ relative to clean air (CA) controls.

Source: Adapted from Heinrich et al., 1989.

the diesel engine emissions. These data are consistent with the diesel exhaust-induced 16 percent increase in pulmonary squamous cell carcinoma in rats previously reported by Heinrich et al. (1986a)

7.2.3 Dermal Application, Subcutaneous Injection, and Intraperitoneal Injection Studies

Kunitake et al. (1986) reported a dose-related response in squamous cell papilloma formation for DET (acetone extract of diesel exhaust tar) and DET + B[a]P applied dermally to female ICR mice (50/group). The diesel exhaust tar was obtained from a heavy-duty (HD) 11-L V-6 engine. A tumor incidence rate of 18 percent was noted for DET (45 mg) + B[a]P (1.8-μg), and an incidence rate of 8 percent was noted for DET (45 mg) alone. No skin cancer was observed for any of the treated mice. In subcutaneous injection experiments, five subcutaneous injections of DET (500 mg/kg per injection) resulted in a significant ($p < 0.01$) increase (22.7 percent) in subcutaneous tumors for female C57Bl mice ($n = 22$). Five subcutaneous doses of DET at 10, 25, 30, 100, or 200 mg/kg failed to produce a significant increase in tumor incidence. One of 12 female mice (8.3 percent) and 4 of 12 male mice (33.3 percent) developed malignant lymphomas following neonatal subcutaneous administration of 10 mg DET/mouse. The malignant lymphoma incidence for the male mice was statistically significant at ($p < 0.05$) from controls. Dermal painting of DET did not induce skin cancer, although multiple subcutaneous administrations of DET at high dose levels (500 mg/kg) produced a significant increase in subcutaneous tumors in mice. Additional studies using LD engine particulate extract dermally applied to female ICR mice (50/group) and subcutaneous injection studies of HD and LD extracts using female ICR and nude mice (BALB/c/cA/JCL-nu) were also reported (Kunitake et al., 1988). In the LD extract dermal exposure experiments, groups of 50 mice were treated with total doses of 45, 15, 5, or 0.5 mg of LD particulate extract. and one control group was given acetone. The LD extract served as an initiator and was applied 10 times every other day. A promoter [2.5 μg of tetradecanoyl phorbol acetate (TPA)] was applied three times a week for 25 consecutive weeks following application of the LD extract. No significant difference was noted between the control group and treatment groups for the incidence of malignant

tumors, papillomas, papillomatous lesions, and acanthosis. In the subcutaneous injection studies, groups of 30 ICR and nude mice were given a single subcutaneous injection of 10 mg HD extract, 10 mg HD + 50 μg TPA, 10 mg LD extract, 10 mg LD extract + 50 μg TPA, or 50 μg TPA. No malignant tumors or papillomas were observed. One papillomatous lesion was observed in an ICR mouse receiving LD extract + TPA, and acanthosis was observed in one nude mouse receiving only TPA.

In what appears to be an extension of the Kunitake et al. (1986) subcutaneous injection studies, Takemoto et al. (1988) presented additional data for subcutaneously administered diesel particulate extracts from HD and LD diesel engines. In this report, the extracts were administered to 5-week old and neonatal (<24-h old) C57Bl mice of both sexes. Exhaust particle extract from HD or LD engines was administered weekly for 5 weeks at doses of 10, 25, 50, 100, 200, or 500 mg/kg with group sizes ranging from 15 to 54 animals. After 20 weeks, comparison with a control group indicated a significant increase in the incidence of subcutaneous tumors for the 500-mg/kg HD group [5 of 22 mice (22.7 percent), $p < 0.01$], the 100 mg/kg LD group [6 of 32 (18.8 percent), $p < 0.01$], and the 500 mg/kg LD group [7 of 32 (21.9 percent), $p < 0.01$] in the adult mouse experiments. The tumors were characterized as malignant fibrous histiocytomas and no tumors were observed in other organs. In the neonate experiments, there was a significantly higher incidence of malignant lymphomas in males receiving HD extract at 10 mg/kg, of lung tumors for males given 2.5 mg HD extract/kg, and for males given 5 mg and females given 10 mg LD extract/kg. A dose-related, but not significant, trend was observed for the incidences of liver tumors for both the HD- and LD-treated neonatal mice. The incidence of mammary tumors in female mice and multiple organ tumors in male mice was also greater for some extract-treated mice but were not dose related. The report concluded that LD particulate extract showed greater carcinogenicity than did HD particulate extract.

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of i.p. injections of diesel exhaust particulate matter on male Strong A mice. Three groups of 30 mice were injected with 0.1 mL of suspension (particles in distilled water) containing 47, 117, or 235 μg of diesel exhaust particles collected from Fluoropore filters in the inhalation exposure chambers. Vehicle controls received injections of particle suspension made from

particulate matter from control exposure filters, positive controls received 20 mg of urethan, and negative controls received no injections. Injections were made three times weekly for 8 weeks resulting in a total diesel particle dose of 1.1, 2.8, and 5.6 mg for the low, medium and high dose groups and 20 mg of urethane for the positive control group. These animals were sacrificed after 26 weeks and examined for lung tumors. For the low, medium, and high diesel exhaust particle dose groups, the tumor incidence was 2/30, 10/30, and 8/30, respectively. The tumor incidence or number of tumors per mouse for the diesel exhaust-treated animals was not significantly different from vehicle controls (8/30) or negative controls (7/28). The urethane-treated (positive) controls had a tumor incidence of 29/29.

The effects of dermal application of extract from diesel exhaust particles was examined by Kotin et al. (1955). Acetone extracts were prepared from a blocked diesel engine (type and size not provided) operated at warm-up mode and under load. These extracts were applied dermally, three times weekly to male and female C57 and Strain A mice. Results of these experiments are summarized in Table 7-22. In the initial experiments using 52 (12 male, 40 female) C57 mice treated with exhaust extract from an engine operated in a warm-up mode, two papillomas were detected after 13 mo. Four tumors in 8 surviving of 50 exposed male Strain A mice treated with exhaust extract from an engine operated under full load were detected 16 mo after the start of treatment. For female Strain A mice treated with extract from an engine operated under full load, 17 tumors were detected in 20 of 25 mice surviving longer than 13 mo. This provided a significantly increased tumor incidence of 85 percent.

Pepelko and Peirano (1983) described a series of experiments wherein male and female Strain A mice were injected with diesel emission particles, particle extracts, or various environmental mixtures of known carcinogenicity including cigarette smoke condensate, coke oven emissions, and roofing tar emissions. Injection of urethan or DMSO served as positive or vehicle controls, respectively. Diesel exhaust was produced by Nissan engines as described for Pepelko and Peirano (1983). An 8-cylinder Oldsmobile engine operated at the equivalent of 40 mph was also used for comparison of emission effects from different makes and models of diesel engine. The mice were sacrificed at 9 mo of age and lungs examined for histopathological changes. The only significant findings other than for positive controls

TABLE 7-22. TUMORIGENIC EFFECTS OF DERMAL APPLICATION OF ACETONE EXTRACTS OF DIESEL EXHAUST

Date started	Number of animals	Strain/sex	Sample material	Date of appearance of first tumor	Survivors at time of first tumor	Total tumors	Date experiment terminated
3/5/52	52	C57/40F, 12M	Extract of particulate material obtained during warm-up	4/1/53	33	2	1/4/54
5/19/52	50	A/M	Extract of particulate material obtained during full load	9/2/53	8	4	4/19/54
2/13/53	25	A/F	Extract of particulate material obtained during full load	3/20/54	20	17	7/23/54

Source: Kotkin et al, 1955.

were increases in numbers of lung adenomas per mouse, and small increases in tumor incidence in male mice receiving Nissan diesel engine exhaust extract and in female mice receiving coke oven extract injections. The number of mice with tumors was not, however, significantly changed in these groups. The overall negative findings of this study may indicate that the lung was not the target organ for the carcinogens present, the active component(s) was not reaching the target organ (lung), or the mouse strain used was not a sensitive test for the type of carcinogens present.

Depass et al. (1982) examined the potential of diesel exhaust particles and dichloromethane extracts of diesel exhaust particles to act as complete carcinogens, carcinogen initiators, or carcinogen promoters. In skin-painting studies, the exhaust material was obtained from an Oldsmobile 350D engine operated under constant load at 65 km/h. The exhaust particles were collected at a temperature of 100±10°C. Groups of 40 C3H/HeJ mice were used because of their low spontaneous tumor incidence. For the complete carcinogenesis experiments, diesel exhaust particles were applied as a 5- or 10 percent suspension in acetone. Dichloromethane extract was applied as 5-, 10-, 25-, or 50 percent suspensions. Negative controls received acetone, and positive controls received 0.2 percent B[a]P. For tumor promotion experiments, a single application of 1.5 percent B[a]P was followed by repeated applications of 10 percent diesel particle suspension, 50 percent diesel particle extract, acetone only (vehicle control), 0.0001 percent phorbol 12-myristate 13-acetate (PMA) as a positive control, or no treatment (negative control). For the tumor initiation studies, a single initiating dose of 10 percent diesel particle suspension, 50 percent diesel particle extract, acetone, or PMA was followed by repeated applications of 0.0001 percent PMA. Following 8 mo of treatment, the PMA dose in the initiation and promotion studies was increased to 0.01 percent. Animals were treated three times per week in the complete carcinogenesis and initiation experiments and five times per week in promotion experiments. All test compounds were applied to a shaved area on the back of the mouse.

In the complete carcinogenesis experiments, one mouse receiving the high (50 percent) suspension developed a squamous cell carcinoma after 714 d of treatment. Tumor incidence in the B[a]P group was 100 percent and no tumors were observed in any of the other groups. Squamous cell carcinomas with pulmonary metastases were identified

in one mouse of the 50 percent diesel exhaust particle extract group. One mouse in the 25 percent extract group developed a grossly diagnosed papilloma. Nineteen positive control mice possessed tumors (11 papillomas, 8 carcinomas). No tumors were observed for any of the other treatment groups. Three tumors (two papillomas and one carcinoma) were identified in the group receiving diesel particle suspension and three tumors (two papillomas and one fibrosarcoma) were found in the diesel exhaust particle extract group. These finding were reported to be statistically insignificant using the Breslow and Mantel-Cox tests.

The data from this study indicated that diesel exhaust particles and dichloromethane extracts of these particles are not effective with regard to tumor promotion or initiation. Although these findings were not consistent with those of Kotin et al. (1955), the occurrence of a single carcinoma in a strain known to have an extremely low spontaneous tumor incidence may be of importance. Furthermore, a comparison between studies employing different strains of mice with varying spontaneous tumor incidences may result in erroneous assumptions.

Nesnow et al. (1982) studied the formation of dermal papillomas and carcinomas following dermal application of dichloromethane extracts from coke oven emissions, roofing tar, diesel engine exhaust, and gasoline engine exhaust. Diesel exhaust from five different engines including a pre-production Nissan 220C, a 350 in^3 Oldsmobile, a prototype VW Turbo Rabbit, a Mercedes 300D, and a heavy-duty Caterpillar 3304 were used for various phases of the study. Male and female Sencar mice (40 per group) were used for tumor initiation, tumor promotion, and complete carcinogenesis studies. For the tumor initiation experiments, the diesel exhaust extracts were topically applied in single doses of 100, 500, 1000 or 2000 μg/mouse. The high dose (10,000 μg/mouse) was applied in five daily doses of 2000 μg. One week later, 2 μg of the tumor promoter, teradecanoylphorbol-acetate (TPA), was applied topically, twice weekly. The tumor promotion experiments used mice treated with 50.5 μg of B[a]P followed by weekly (twice weekly for high dose) topical applications (at the aforementioned doses) of the extracts. For the complete carcinogenesis experiments, the test extracts were applied weekly (twice weekly for the high doses) for 50 to 52 weeks. Only extracts from the Nissan, Oldsmobile, and Caterpillar engines were used in the complete carcinogenesis experiments.

In the tumor initiation studies, both B[a]P alone and the application of the Nissan engine exhaust extract followed by TPA treatment produced a significant increase in tumor (dermal papillomas) incidence at 7 to 8 weeks postapplication. By 15 weeks, the tumor incidence was greater than 90 percent for both groups. No significant carcinoma formation was noted for mice in the tumor initiation experiments following exposure to the extracts of the other diesel engines, although the Oldsmobile engine exhaust extract at 2.0 mg/mouse did produce a 40-percent papilloma incidence in male mice at 6 mo. This effect was, however, not dose-dependent.

Results of the tumor promotion experiments indicated that coke oven extract and roofing tar were as effective as the TPA positive control at promoting B[a]P-initiated papillomas. No diesel exhaust extracts were tested for tumor promotion activity.

For complete carcinogenesis activity, B[a]P (50.5 μg/week), coke oven extract (at 1.0, 2.0, or 4.0 mg/week), and the highest dose of roofing tar extract (4.0 mg/week) all tested positive. Exhaust extracts from only the Nissan, Oldsmobile, and Caterpillar engines were tested for complete carcinogenic potential, and all three proved to be negative using the Sencar mouse assay.

The results of the dermal application experiments by Nesnow et al. (1982) are presented in Table 7-23. The tumor promotion-initiation assay was considered positive if a dose-dependent response was obtained and if at least two doses provided a papilloma-per-mouse value that was three times or greater than that of the background value. Based on these criteria, only emissions from the Nissan were considered positive. Tumor initiation and complete carcinogenesis assays required that at least one dose produce a tumor incidence of at least 20 percent. None of the diesel exhaust samples yielded positive results based on this criterion.

7.2.4 Summary of Animal Carcinogenicity Studies

As early as 1955, Kotkin et al. (1955) provided evidence for tumorigenicity and carcinogenicity of acetone extracts of diesel exhaust and also provided data suggesting a difference in this potential depending on engine operating mode. Until the mid 1980s, no

TABLE 7-23. DERMAL TUMORIGENIC AND CARCINOGENIC EFFECTS OF VARIOUS EMISSION EXTRACTS

	Tumor initiation		Complete carcinogenesis	Tumor promotion
Sample	Papillomas[a]	Carcinomas[b]	Carcinomas[b]	Papilomas[a]
Benzo[a]pyrene	+/+[c]	+/+	+/+	+/+
Topside coke oven	+/+	-/+	ND[d]	ND
Coke oven main	+/+	+/+	+/+	+/+
Roofing tar	+/+	+/+	+/+	+/+
Nissan	+/+	+/+	-/-	ND
Oldsmobile	+/+	-/-	-/-	ND
VW Rabbit	+/+	-/-	I[e]	ND
Mercedes	+/-	-/-	ND	ND
Caterpillar	-/-	-/-	-/-	ND
Residential furnace	-/-	-/-	ND	ND
Mustang	+/+	-/+	ND	ND

[a]Scored at 6 months
[b]Cumulative score at 1 yr.
[c]Male/female.
[d]ND = Not determined.
[e]I = Incomplete.

Source: Nesnow et al, 1982

chronic studies assessing inhalation of diesel exhaust, the relevant mode for human exposure, had been reported, and it is this route of exposure with which the most extensive, recent studies were concerned. Studies employing rats and an adequate experimental design were nearly all positive in demonstrating diesel exhaust-induced increases in tumorigenicity. The 9.5 percent increase in tumor incidence for female Wistar rats reported by Heinrich et al. (1986a) is supported by the report by Mauderly et al. (1987), which reported a 3.6-percent and 12.8-percent tumor incidence in F344 rats following chronic exposure to diesel exhaust at particle concentrations of 3.5 and 7.0 mg/m^3, respectively. However, only one of the squamous cell tumors reported by Heinrich et al. (1986a) was classified as a carcinoma. In the Mauderly et al. (1987) study, the carcinoma incidence was 0.9, 1.3, 0.5, and 7.5 percent for the control, low, medium, and high exposure groups, respectively. The study by Wong et al. (1986) affirms observations of potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. Similarly, Iwai et al. (1986), demonstrated diesel exhaust-induced tumorigenicity in rats exposed to an exhaust particle concentration of 4.9 mg/m^3, although the sample size was small. This study also reported development of a splenic lymphoma which represents the only nonpulmonary tumor resulting from inhalation exposure to diesel exhaust. The long-term study by Ishinishi et al. (1986) showed a greater incidence of carcinomas (6.5 percent) in rats following 30-mo exposure to diesel exhaust at 4 mg/m^3, but not at lower (0.4-, 1-, or 2-mg/m^3) exposure levels. However, Brightwell et al. (1986) demonstrated a dose-dependent increase in tumor incidence for male and female F344 rats exposed to unfiltered diesel exhaust (five 16-h periods per week) at concentrations as low as 2.2 mg/m^3 and also at 6.6 mg/m^3. This study indicated that the tumor incidence was higher for female (0 percent, 15 percent, or 54 percent at 0.0, 2.2, or 6.6 mg/m^3) than for male rats (1 percent, 4 percent, or 23 percent for 0.0, 2.2, or 6.6 mg/m^3). Thus, these studies demonstrated carcinogenic effects in rats at exposure levels ranging from 2.2 to 7.0 mg/m^3. Heinrich et al. (1986a,b) and Pepelko and Peirano (1983) also provided evidence of carcinogenicity in mice.

Both the Heinrich et al. (1986a) and Brightwell et al. (1986) studies provided negative results for tumorigenicity of diesel exhaust in hamsters, a species known for its resistance to tumor induction. Negative results were also presented by several other investigators

(Takemoto et al., 1986; Schreck et al., 1981; Karagianes et al., 1981), but these studies tended to employ inadequate exposure durations, low exposure concentrations, or inadequate animal numbers per group. Similarly, the studies using monkeys and cats were of inadequate duration (2 yr) for these longer-lived species.

Alternate exposure routes including dermal exposure, skin painting, and subcutaneous injection provided additional evidence for tumorigenic effects of diesel exhaust. Evidence for tumorigenicity was demonstrated by Kotin et al. (1955) for mice to which an acetone extract of diesel exhaust particles was applied dermally. Nesnow et al. (1982) also showed that extracts from some diesel engines were potentially tumorigenic following dermal application to rodents. A significant increase in the incidence of subcutaneous tumors in female C57Bl mice was reported by Kunitake et al. (1986) for subcutaneous administration of light-duty diesel exhaust tar extract at doses of 500 mg/kg. Doses at or below 200 mg/kg, however, were negative. Takemoto et al. (1988) provided additional data for this study and reported an increased tumor incidence in the mice following injection of light-duty engine exhaust extract at doses of 100 and 500 mg/kg. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of diesel exhaust particle suspensions. However, in this study the exhaust particles were collected at temperatures of 100 °C, a temperature that would minimize the condensation of vapor-phase organics and, therefore, reduce the availability of potentially carcinogenic compounds that might normally be present on diesel exhaust particles. Intraperitoneal injection studies were generally negative.

Experiments using tumor initiators such as DEN, B[a]P, DPIN, or DBahA (Brightwell et al., 1986; Heinrich et al., 1986a; Takemoto et al., 1986) did not provide conclusive results regarding the carcinogenic potential of filtered vs. whole diesel exhaust. Some of these studies, however, strongly imply the importance of adsorbed organic compounds for the carcinogenic effect, and data are available regarding the carcinogenic activity of several compounds known to be components of diesel exhaust.

Diesel exhaust is composed of gaseous and particulate phases and is known to be a complex mixture containing verified and potential carcinogens. A study by Grimmer et al. (1987) demonstrated that a whole exhaust condensate fraction (IIb) containing PAHs with

4 to 7 rings produced a high tumor incidence when implanted into rat lungs. It was also noted that this fraction represented only 0.8 percent of the total weight of the exhaust condensates, and that some tumorigenicity was also associated with nitroaromatic fractions. The PAH fraction produced a tumor incidence similar to that of a low concentration of B[a]P.

An additional point of concern, as of yet unresolved, is the role of the diesel exhaust particle in carcinogenicity. Several of the previously discussed studies indicated that only whole (unfiltered) diesel exhaust is tumorigenic or carcinogenic and that these properties are eliminated or greatly minimized in filtered diesel exhaust exposure. Although the tumorigenicity of the gaseous fraction is presently unresolved and experiments using filtered exhaust were negative, most of these experiments did not provide definitive evidence that a maximum tolerated dose was achieved. Furthermore, it has not yet been unequivocally determined that the carbon core of the exhaust particle is not without carcinogenic potential. The fact that allegedly inert, "noncarcinogenic" dusts such as titanium dioxide, shale dust, and quartz dust have been shown to induce lung cancer at very high concentrations is of concern in this respect. That the carbon core alone may be carcinogenic or may have promotional effects in initiated cells remains to be determined. Studies currently in progress may resolve this question.

Although uncertainties exist regarding the tumorigenic potential of the gaseous component and the carbon core component of diesel exhaust, it is clear that diesel exhaust is carcinogenic in animals. This contention is supported by positive results in numerous, independent studies in male and females of at least two species and by several routes of administration, including inhalation, intratracheal administration, skin painting, and subcutaneous injection.

A summary of studies assessing the tumorigenic and carcinogenic effects in laboratory animals following inhalation exposure to diesel exhaust is presented in Table B-1 of Appendix B.

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8. PHARMACOKINETIC CONSIDERATIONS IN THE PULMONARY CARCINOGENICITY OF DIESEL ENGINE EMISSIONS

8.1. INTRODUCTION

Analysis of whole animal studies examining the carcinogenicity of diesel exhaust revealed the importance of understanding dosimetric factors in evaluating the carcinogenic potential of diesel emissions. Several studies addressed the effects of diesel exhaust particulate matter concentration on the pulmonary retention, clearance, and the potential for pulmonary overload of these particles. Although many other factors, such as metabolism considerations and target organ sensitivity, are important in obtaining an accurate assessment of risk, understanding aspects of pulmonary retention, clearance and bioavailability of potentially carcinogenic compounds are also quite relevant. The following sections summarize those studies examining pharmacokinetic parameters such as exposure concentration, clearance rates and compartmental considerations, and alveolar macrophage functions as well as factors possibly affecting these processes and the ramifications of these regarding the bioavailability of particle-associated organics. Additionally, some discussion of factors deemed to be important in extrapolation modeling to humans will be discussed to the extent feasible.

8.2. REGIONAL LUNG DEPOSITION OF INHALED PARTICLES BY HUMANS AND ANIMALS

The site of deposition of particulate matter in the respiratory tract is dependent upon a number of factors including respiratory tract anatomy (airway dimensions and branching configurations) and physiology (breathing mode and rate, pulmonary volumes and capacities, and clearance mechanisms) and the physicochemical characteristics (particle size, shape, density, and solubility) of the inhaled particles. Deposition of particles may occur through several different processes or combinations of these, including diffusion, sedimentation,

interception, electrostatic precipitation, and impaction. Obviously, the anatomical and physiologic factors are paramount in determining dissimilar regional deposition, especially among species. A large airway diameter creates a greater turbulent flow velocity than does a small airway diameter. Assuming the physicochemical characteristics of the particle to be constant, it follows that the larger diameter airways of humans would create a turbulent flow and the smaller diameter airways of laboratory animals such as rodents would exhibit a laminar flow. Furthermore, when compared with humans, most laboratory animals have tracheas that are longer relative to the diameter. The flow patterns resulting from these anatomical differences would greatly affect most of the deposition processes. For example, increased flow through the larger diameter primary passages of humans would result in greater deposition of particles in the bronchi. Humans also tend to have a more symmetrical branching pattern than do animals. a condition that results in greater impaction deposition at the carinae of the bronchi and bronchioles. Factors affecting pulmonary deposition are reviewed in U.S. EPA (1988) and for more detailed consideration of these processes, the reader is referred to Raabe (1979), Phalen and Oldham (1983), and Lippmann and Schlesinger (1984).

8.3. TRACHEOBRONCHIAL CLEARANCE OF PARTICULATE MATTER

Upon inhalation of particulate matter. deposition will initially occur in the nasopharyngeal and tracheobronchial regions of the respiratory tract. Due to high air flow velocities and abrupt directional changes in these regions. inertial impaction and sedimentation are the primary deposition mechanisms (especially for particles larger than 2.5 μm aerodynamic equivalent diameter). Mechanical processes such as sneezing and nose wiping remove particles from the nasopharyngeal region. Clearance of particulate matter from the tracheobronchial region is mediated primarily by mucociliary transport, and is usually a more rapid clearance process than those operating in deep lung regions. Mucociliary transport (often referred to as the mucociliary escalator) is accomplished by the mucus-covered, ciliated epithelia which line the respiratory tract from the trachea through the terminal bronchioles. Clearance rate by this system is determined primarily by the flow velocity of

the mucus, which is greater in the proximal airways and decreases distally. These rates also exhibit interspecific variability. The half-times for tracheobronchial clearance are usually on the order of hours while those for pulmonary clearance may be hundreds of days or years. The relationship between the early clearance of insoluble particles from tracheobronchial regions and their long-term clearance from pulmonary regions is illustrated in Figure 8-1.

Cuddihy and Yeh (1986) reviewed respiratory tract clearance of particles inhaled by humans. Depending on the type of particle (ferric oxide, teflon discs, albumin microspheres), the technique employed, and the anatomic region (midtrachea, trachea, main bronchi), particle velocity (moved by mucociliary transport) ranged from 2.4 to 21.5 mm/min. The highest velocities were recored for midtracheal transport, and the lowest were for main bronchi. In one study, an age difference was noted for tracheal mucociliary transport velocity (5.8 mm/min for individuals less than 30 years of age, and 10.1 mm/min for individuals over 55 years of age).

The Cuddihy and Yeh (1986) report described salient points to be considered when estimating particle clearance velocities from tracheobronchial regions and included: respiratory tract airway dimensions, calculated inhaled particle deposition fractions for individual airways, and thoracic clearance measurements. Predicted clearance velocities for the trachea and main bronchi were found to be similar to those experimentally determined for inhaled radiolabeled particles but not for intratracheally instilled particles. Figure 8-2 illustrates a comparison of the short-term clearance of inhaled particles by human subjects and the model predictions for this clearance.

8.4. CLEARANCE FROM DEEP LUNG REGIONS

Several studies have investigated the effects of exposure concentration and lung burden on the pulmonary clearance of diesel exhaust particles by laboratory animals. These factors are important for establishing dose-response relationships and determining the bioavailability of organic compounds adsorbed to these particles.

Wolff et al. (1986, 1987) provided clearance data ($t_{1/2}$) and lung burden values for F344 rats exposed to diesel exhaust for 7 h/d, 5 d/week for 24 mo. Exposure

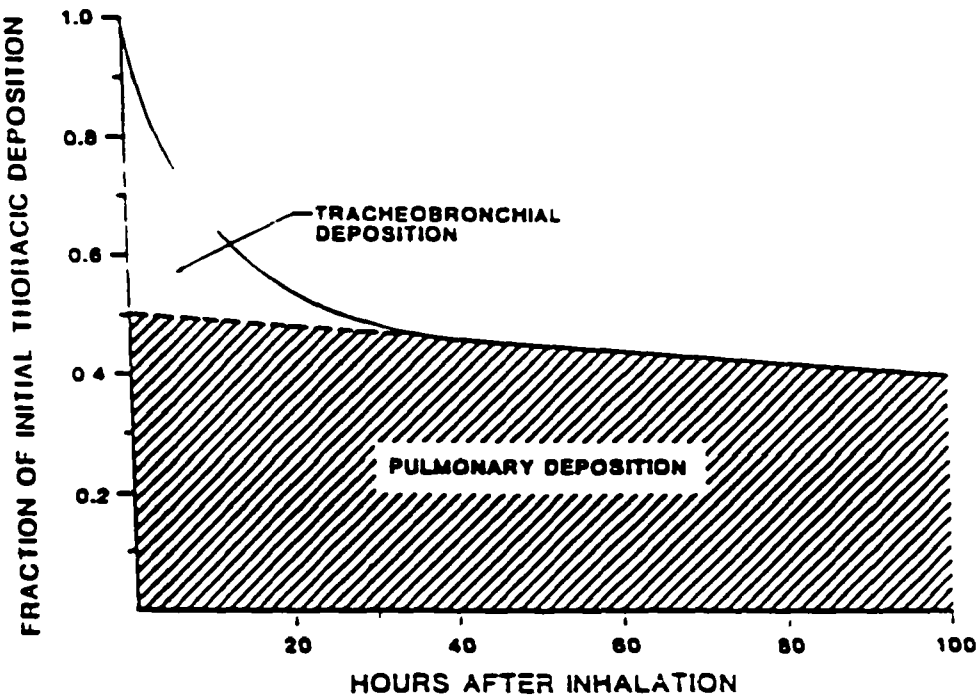

Figure 8-1. Clearance of insoluble particles deposited in tracheobronchial and pulmonary regions.

Source: Cuddihy and Yeh, 1986.

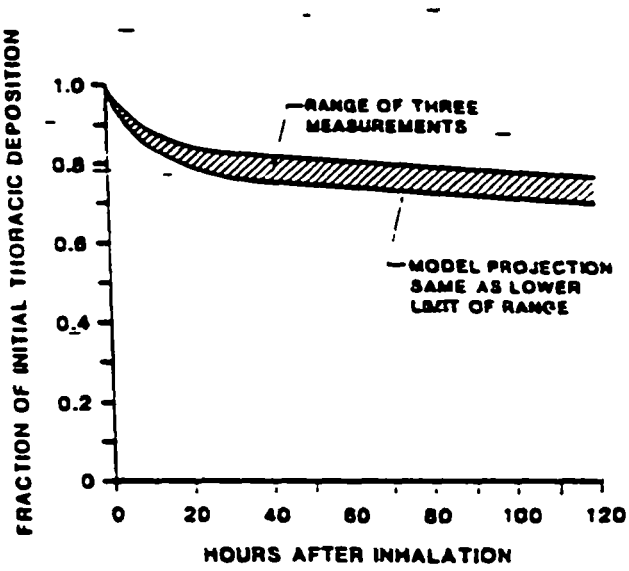

Figure 8-2. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

Source: Cuddihy and Yeh. 1986 (from Stahlhofen et al., 1980)

concentrations of 0.35, 3.5, and 7.0 mg soot/m^3 were employed in this whole body-inhalation exposure experiment. Intermediate clearance of $^{67}Ga_2O_3$ particles (one half-hour, nose-only inhalation) was assessed after 6, 12, 18, and 24 mo of exposure at all of the diesel exhaust concentrations. A two-component function described the clearance of the administered radiolabel:

$$F_{(t)} = A \exp(-0.693\ t/\tau_1) + B \exp(-0.693\ t/\tau_2)\ ,$$

where F(t) was the percentage retained, A and B were the magnitudes of the two components [nasal, pulmonary conducting airways, and gastrointestinal clearance], and intermediate lung clearance, respectively], and τ_1 and τ_2 the half-times for the A and B components, respectively. The early clearance half-times (τ_1), representing clearance from primary, ciliated conducting airways, were similar for rats in all exposure groups at all time points except for those in the high exposure (7.0 mg/m^3) group following 24 mo of exposure where the clearance time was faster than that of the controls. Significantly longer B component clearance half-times, representing the more rapid clearance from nonciliated passages such as bronchioles and alveoli, were noted after as few as 6 mo exposure to diesel exhaust at 7.0 mg/m^3 and 18 mo exposure to 3.5 mg/m^3. Nose-only exposure to ^{134}Cs fused aluminosilicate particles (FAP) were used to assess long-term clearance. Following 24-mo exposure to diesel exhaust, long-term clearance of ^{134}Cs-FAP was significantly ($p < 0.01$) altered in the 3.5 and 7.0 mg/m^3 exposure groups ($t_{1/2}$ of 264 and 240 d, respectively) relative to the 0.35 mg/m^3 and control groups ($t_{1/2}$ of 81 and 79 d, respectively). Long-term clearance represents the slow component of particle removal from alveoli. The decreased clearance correlated well with increased lung burden in the 3.5 and 7.0-mg/m^3 exposure groups.

The Heinrich et al. (1986) and Stober (1986) studies reported increased tumor incidence following lifetime inhalation exposure to diesel exhaust (4 mg/m^3) for 19 h/d, 5 d/week. The impaired pulmonary clearance observed in the rats exposed to total diesel exhaust may have allowed for greater desorption of particle-bound PAHs, thus accounting for the increased tumor incidence. Based on the analysis of the diesel exhaust used in the experiments, values for rat ventilatory functions, and estimates of deposition and clearance,

bioavailability of particle-bound organic material was estimated at 6 to 15 mg for a 2.5 yr period.

Chan et al. (1981) subjected 24 male F344 rats to nose-only inhalation of diesel exhaust (6 mg/m^3) labeled with ^{131}Ba or ^{14}C for 40 to 45 minutes, and assessed total lung deposition, retention, and elimination. The deposition efficiency based on radiolabel inventory was 15 to 17 percent. Quantitation of ^{131}Ba label in the feces during the first 4 d following exposure indicated that 40 percent of the diesel particulates were eliminated via mucociliary clearance. Clearance of the particles from the lung followed a two-phase elimination described by:

$$R(t) = Ae^{k1t} + Be^{-k2t}$$

where the first term represents a rapid ($t_{1/2}$ of 1 d) mucociliary transport elimination, and the second term represents a slower ($t_{1/2}$ of 62 d) macrophage-mediated alveolar clearance. The effects of prolonged exposure and overloading were not addressed in this study.

Accumulated lung burden of diesel soot particles following 18 mo, 7 h/d, 5 d/week exposure to diesel exhaust was reported by Griffis et al. (1983). Male and female F344 rats exposed to 0.15, 0.94, or 4.1 mg soot/m^3 were sacrificed at 1 d, and 1, 5, 15, 33, and 52 weeks after exposure, and diesel soot extracted from lung tissue dissolved in tetramethylammonium hydroxide. Following centrifugation and washing of the supernatant, diesel soot content of the tissue was quantitated using spectrophotometric techniques. The analytical procedure was verified by comparing results to recovery studies using known amounts of diesel soot in lungs of unexposed rats. Lung burdens of the 0.15, 0.94, and 4.1 mg soot/m^3 exposure groups were 35, 220, and 1890 μg/g lung, respectively, 1 d after the 18-week exposure. Long-term clearance rates for the 0.15 and 0.94 mg/m^3 groups had estimated half-times of 87±28 and 99±8 d, respectively. The clearance half-time for the 4.1 mg/m^3 exposure group was 165±8 d which was significantly (p <0.0001) greater than those of the lower exposure groups. This study provided evidence for an overload phenomenon and possible threshold relative to clearance of diesel exhaust particulates from the lungs of rats.

In a subsequent study (Lee et al., 1983) a three-phase model was used to describe the clearance of diesel exhaust particles (7 mg/m^3 for 45 min or 2 mg/m^3 for 140 min) by F-344 rats (24 per group) exposed by nose-only inhalation. The exposure protocols provided comparable total doses based on ^{14}C radiolabel. $^{14}CO_2$ resulting from combustion of ^{14}C-labeled diesel fuel was removed by a diffusion scrubber to avoid erroneous assessment of ^{14}C intake by the animals. Retention of the radiolabeled particles was determined up to 335 d after exposure, and resulted in the derivation of a three phase clearance of the particles. The resulting clearance half-times for the three-phases were 1, 6, and 80 d. The three clearance phases are taken to represent removal of tracheobronchial deposits by the mucociliary escalator, removal of particles deposited in the proximal respiratory bronchioles, and alveolar clearance, respectively. Species variability in clearance of diesel exhaust particles was also demonstrated by the fact that Hartley guinea pigs exhibited negligible clearance from day 10 to day 432 following a 45 min exposure to a diesel particle concentration of 7 mg/m^3. Initial deposition efficiency (20±2 percent) and short-term clearance were, however, similar to the rats.

Lung clearance in male F344 rats pre-exposed to diesel exhaust at 0.25 or 6 mg/m^3, 20 h/d, 7 d/week for periods lasting from 7 to 112 d was studied by Chan et al. (1984). Following this pre-exposure protocol, rats were subjected to 45 min nose-only exposure to ^{14}C-diesel exhaust, and pulmonary clearance of radiolabel monitored for up to 1 yr. First order clearance for the two compartment model, $R(t) = Ae^{-a1t}+Be^{-a2t}$, yielded alveolar clearance half-times of 166 and 562 d for rats pre-exposed to 6.0 mg/m^3 for 7 and 62 d, respectively. These values were significantly ($p < 0.05$) greater than the clearance half-time of 77±17 d for control rats. The same clearance values for rats of the 0.25-mg/m^3 groups were 90±14 and 92±15 d, respectively. Estimated lung burdens of diesel exhaust particles for controls, and for those exposed for 7 d and 62 d to 6.0 mg/m^3 were 0, 0.7 and 6.5 mg, respectively. Lung burden of particulates resulting from pre-exposure to 6.0 mg/m^3 for 112 d was 11.8 mg. No alveolar clearance was observed for these rats. The two-compartment model, however, represents overall clearance of the tracer particles and does not account for a slower clearing compartment for particles sequestered in alveolar macrophage aggregates. A lung retention model for pre-exposed rats accounts for mucociliary clearance

(1st term), an active clearance phase by alveolar macrophages (2nd term), and a residual fraction representing macrophage aggregates with limited clearance capabilities (3rd term)

$$R(t) = Ae^{-\lambda_1 t} + Be^{-\lambda_2 t} + R_0 .$$

The magnitude of R_0 was shown to be dependent on the initial particulate burden and, therefore, would increase in higher exposure scenarios. This study emphasizes the importance of particle overloading of the lung, and the ramifications on clearance of particulates and associated organics.

Morrow (1988) provided additional information on the significance of alveolar macrophage-mediated clearance of particulates from the lungs and possible mechanisms by which this process becomes overloaded. Based on current data for exposure, deposition rate, pulmonary retention half-time, and ventilatory parameters, kinetic descriptions of particle buildup and steady-state phases are provided. These kinetic models, along with experimentally determined alveolar macrophage pool size for rat lungs (2.5×10^7 cells) and particle size data, provide a basis for describing the overloading of alveolar macrophages when rats are used as the model system. Since histological data indicate that the phagocytic functions of alveolar macrophages are not impaired in the presence of high particulate concentrations, it is postulated that impaired translocation of particle-bound macrophages may be the critical factor in reduced pulmonary clearance of particulates. The precise mechanism by which macrophage translocation is impaired is uncertain but may involve alterations of chemoattractants, a critical volume overload of the macrophage, or alterations of activators and modulators of alveolar macrophage function.

Lehnert (1988), in studying particle-alveolar macrophage relationships during clearance of insoluble, noncytotoxic particles, provided results indicating that phagocytized particles were gradually redistributed among the total lung macrophage population during the clearance process. This type of translocation would affect the ultimate clearance of the particulate matter and, at least in part, may explain long-term retention of particulate matter.

Oberdöerster et al. (1988), using F344 rats receiving intratracheal instillations of 3.3 μm polystyrene microspheres labeled with ^{141}Ce and 10.3 μm particles labeled with ^{95}Nb, followed lung clearance of these particles for 200 d after administration of a high dose (100 μg small particles + 100 μg large particles) or a low does (40 μg small + 10 μg large particles). Only minimal clearance was detected for the large particles at both doses, suggesting inability of alevolar macrophages containing one large particle to clear from the lung. The researchers hypothesized that the reported minimal clearance of the large particles was analogous to a chronic overloading with small particles. It was noted that the volume (600 μm^3) of one large particle (10.3 μm) represented 60 percent of the volume of an average alveolar macrophage and that this critical level could be obtained by chronic overloading with many small particles that would provide an equivalent volume. The study indicated that impairment of alveolar macrophage-mediated clearance of particles would occur when this critical phagocytized volume is maintained for extended periods.

8.4.1. Species Variability in Pulmonary Clearance Processes

The delivered dose of potentially carcinogenic agents in diesel exhaust is dependent upon a number of factors. The net dose received will be dependent upon the minute ventilation and, therefore, a smaller animal may receive a higher total dose of particles because of a higher ventilation rate-to-body weight ratio. Additionally, factors such as pulmonary deposition of particulate matter, total lung burden of particles and their clearance from the lungs, and dissolution of organic compounds from the particulate matter are instrumental in determining the bioavailable dose. Of special significance in this respect is species variability in pulmonary clearance of particle burden and its ramifications in extrapolating from animal data to human risk assessment. This emphasis is due, in part, to the fact that longer clearance times may be associated with increased bioavailability of chemicals adsorbed to the particulate matter. The effects of interspecific variability in long-term pulmonary clearance of particulate matter on dose adjustment have been reviewed by Pepelko (1987).

As indicated in the previous section, pulmonary clearance of particulate matter may occur as a rapid phase (mediated primarily by pulmonary alveolar macrophages) and as a long-term phase. The latter is a function of a several factors total lung burden of particles and overloading, dissolution and absorption, and physical transport. The physical transport processes are predominant in the conducting airways and are discussed more fully in the section on tracheobronchial clearance. The long-term clearance processes are concerned primarily with clearance from alveolar regions of the lung, and it is these processes that may exhibit the greatest interspecific variability.

Pepelko (1987) noted that alveolar clearance rates were similar for dogs and humans but at least four times more rapid in rats. Most other laboratory species were similar to rats with the exception of guinea pigs which cleared aluminosilicate particles at a rate similar to that of humans (Snipes and McClellan, 1984 as reported in Pepelko. 1987).

A wide range of clearance rates for particulate matter has been reported for several animal species and humans (Pepelko, 1987). A review of the data for pulmonary clearance of various compounds by dogs and rats indicated, overall, that the clearance half-time ($t_{1/2}$) for dogs was longer than that for rats. Based on the first few months of pulmonary clearance of aluminosilicate reported by Snipes et al. (1983) for rats ($t_{1/2}$ of 35 d) and by Bailey et al. (1982) for humans ($t_{1/2}$ of 420 d), it appears that the clearance rate for rats may be considerably more rapid. In comparing clearance rates of humans with those of dogs, the $t_{1/2}$ values are similar for iron oxide, but for manganese oxide the $t_{1/2}$ for humans was approximately twice that of dogs (Pepelko, 1987). Additionally, intraspecific variability was also pronounced, especially when comparing particles of different chemical composition and size. A review of clearance half-times for rats indicated that soluble particles were cleared more rapidly than insoluble particles. For example, the $t_{1/2}$ for the relatively soluble compounds, vanadium pentoxide and barium sulfate, was 1.8 to 3.0 d, while the insoluble titanium oxide and carbon core diesel particles had a $t_{1/2}$ ranging from 60 to 87 d.

8.4.2. Role of Alveolar Macrophages in the Clearance of Particulate Matter

8.4.2.1. Alveolar Macrophage-Mediated Clearance of Insoluble Particles from the Lung

Alveolar macrophages (AM) constitute an important, first-line cellular defense mechanism against inhaled particles that deposit in the peripheral regions of the lung. It is well established that a host of diverse materials, including particulate diesel exhaust, are phagocytized by the AM shortly after deposition (White and Garg, 1981; Lehnert and Morrow, 1985), and, in this manner, such cell-contained particles are generally rapidly sequestered from the extracellular fluid lining in the alveolar region and from the potentially sensitive alveolar epithelial cells. In addition to this role in compartmentalizing particles from other lung constituents, the AM are also believed to be prominently involved in mediating the clearance of relatively insoluble particles from the air spaces (Lehnert and Morrow, 1985). Although the details of the actual process have not been delineated, AM with their particulate burdens gain access and become coupled to the "mucociliary escalator" and are subsequently transported from the lung via the conducting airways. While circumstantial in nature, numerous lines of evidence indicate that such AM-mediated particle clearance is normally the predominant mechanism by which relatively insoluble particles are removed from the lungs (Lehnert and Morrow, 1985; Powdrill et al., 1989; Gibb and Morrow, 1962; Ferin, 1982).

The removal characteristics for particles deposited in the lung's alveolar region have been descriptively represented by numerous investigators as a multicompartment or multicomponent process with each component following simple first-order kinetics (Snipes and Clem, 1981; Snipes et al., 1988; Lee et al., 1983). While often times suitable as a mathematical approach for describing lung retention data, actual physiologic mechanisms that form the bases for differing empirically resolvable compartments or components of alveolar phase clearance [i.e., early, more rapid component(s)], and slower, longer term component(s) have not been well characterized. Regardless, it seems reasonable to assume that the lung's population of AM must in some manner play a central role in the overall

retention characteristics of insoluble particles that deposit on the alveolar surface. Delineation of this role under conditions of lung burdens commensurate with typical environmental exposures to particles and following particulate exposure levels that lead to diminutions of the AM-mediated clearance process should provide basic information on the phenomenon of particle overload that has been associated with the development of lung tumors following the deposition of high lung burdens of diesel (Mauderly et al., 1987) as well as other types of particles; for example, titanium dioxide (Lee et al., 1985), silica (Holland et al., 1985), and chromium dioxide (Lee et al., 1988). Additionally, lung dosimetric models could incorporate this information for the purpose of extrapolating from existing animal data involving diesel particle exposures to the human condition.

Lehnert and co-workers (1988, 1989, and presently unpublished observations) recently performed a study using laboratory rats to examine particle-AM relationships over the course of alveolar clearance of low to high lung burdens of noncytotoxic polystyrene microspheres (2.13 mm diam.) as an approach to obtain information on potential AM-related mechanisms that form the underlying bases for kinetic patterns of alveolar clearance as a function of particulate lung burdens. The intratracheally instilled lung burdens studied were 1.6×10^7 particles ($\sim$85 μg) for the low lung burden, 2.0×10^8 particles ($\sim$1.06 mg) for a medium range lung burden, and 6.8×10^8 particles ($\sim$3.6 mg) for the highest lung burden. The retention kinetics of the particles following the deposition of these three lung burdens (Figure 8-3) were described using a two-compartment model of the following form: $LB_{(t)} = Ae^{-\lambda_1 t}+Be^{-\lambda_2 t}$, where $LB_{(t)}$ is the lung burden of particles present at a postinstillation time (t in d), and A and B are the coefficients associated with the retention rates λ_1 and λ_2, respectively. The half-times ($t_{1/2}$) of both the early and latter components of clearance were virtually identical following the deposition of the 85 μg and 1.06 mg lung burdens. The coefficients A and B were also closely similar. [Statistical analyses revealed that the rate constants and the coefficients A and B for the low- and medium-range lung burdens were not significantly different from one another]. For the highest lung burden (3.6 mg), significant prolongations in the rates of the early, more rapid component and the longer term, slower

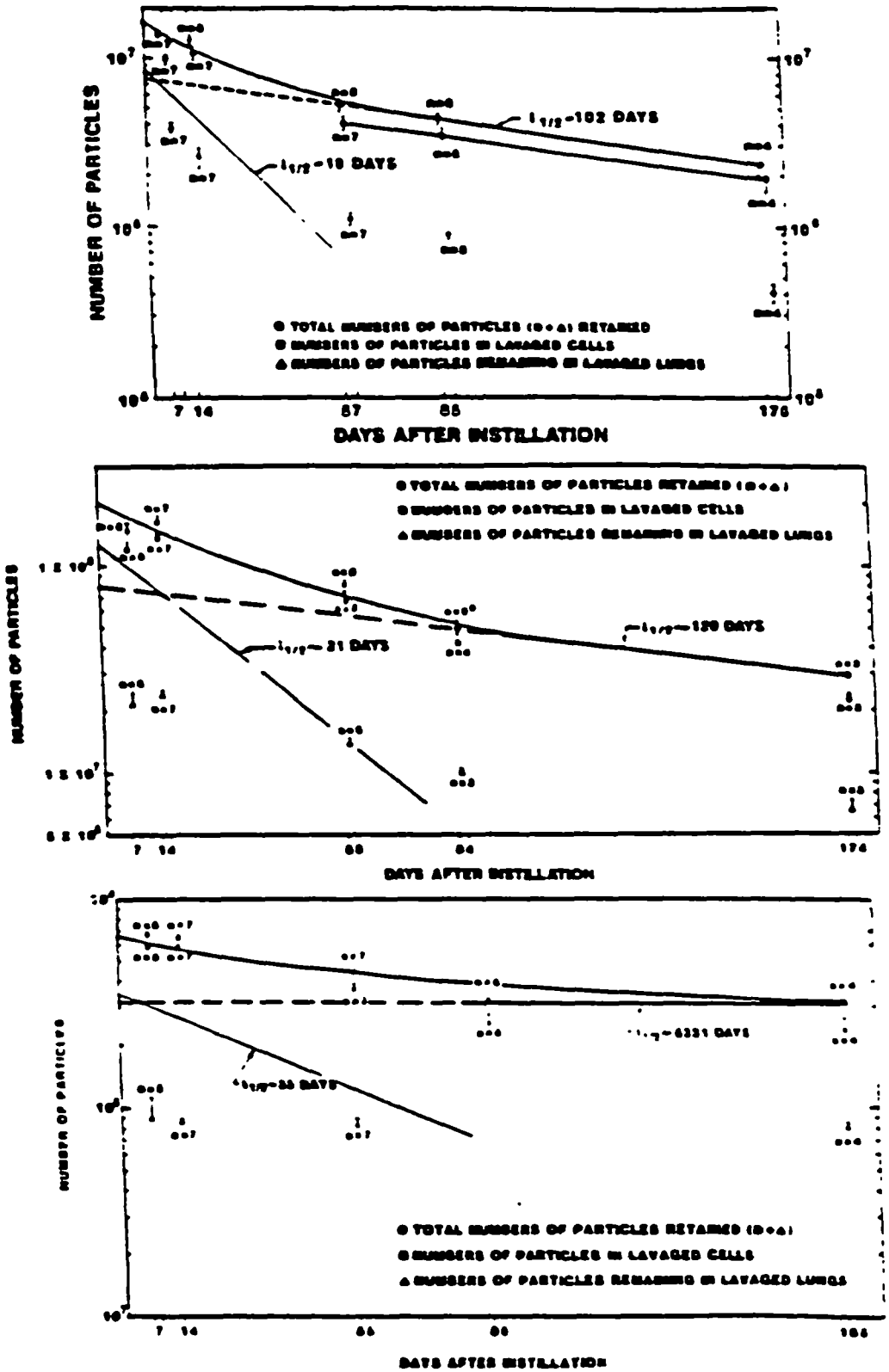

Figure 8-3: Lung retention kinetics of 2.13 mm diam. polystyrene microspheres after instillation into rat lungs. A: 1.6×10^{7} particles; B: 2.0×10^{8} particles; C: 6.8×10^{8} particles.

component of alveolar clearance were found. The percentages of the particle burden associated with the earlier and later components, however, were similar to those of the lesser lung burdens. Particle-AM relationships (based on analyses of lavaged AM) during the clearance of the low lung burden were expressed in terms of the estimated total numbers of AM in the lung that contained a given load of the particles (Figure 8-4). Over a 176-d period following the deposition of the low lung burden, the apparent overall rate(s) of disappearance of AM with engulfed particles was found to increase with increasing particle burdens in the macrophages. For particle burdens up to 13 to 14 microspheres per AM, the disappearance of the AM followed a biphasic pattern, which, like the lung retention data, could be described as a two-component, negative exponential equation. A plot of the later component exponents (*d* in Figure 8-5) relative to AM-particle burdens showed a linear trend between the rate of later phase AM disappearance and particle burden per cell (i.e., the magnitudes of the exponents decreased linearly as the number of microspheres defining an AM category increased). Analyses of the early components of AM disappearance also pointed to decreases in the magnitude of the exponents with increasing burdens in the AM (*b* in Figure 8-6). The proportions of the AM with a given particle burden that disappeared from the total AM population via an apparent early component are graphically summarized in Figure 8-7. Unlike the lower particle burden per AM categories, the disappearance rates for AM in the higher particle categories were found to be best fitted with single exponential functions, the exponents of which appeared to be more akin to the later component rates of the other particle-AM categories than they were to the early component rates of AM with less than 15 particles.

The simplest explanation for the "disappearance" characteristics of AM with differing particle loads seen in the above study is that the varying rates by which AM seemingly vanish from the lung's AM population during the course of alveolar clearance of the particles denotes the actual removal kinetics of these cells from the lung via thetracheobronchial route. This interpretation, accordingly, implies that the translocation of AM from the alveolar space by way of the conducting airways is fundamentally influenced by the particle burdens in the cells so translocated. On the other hand, the

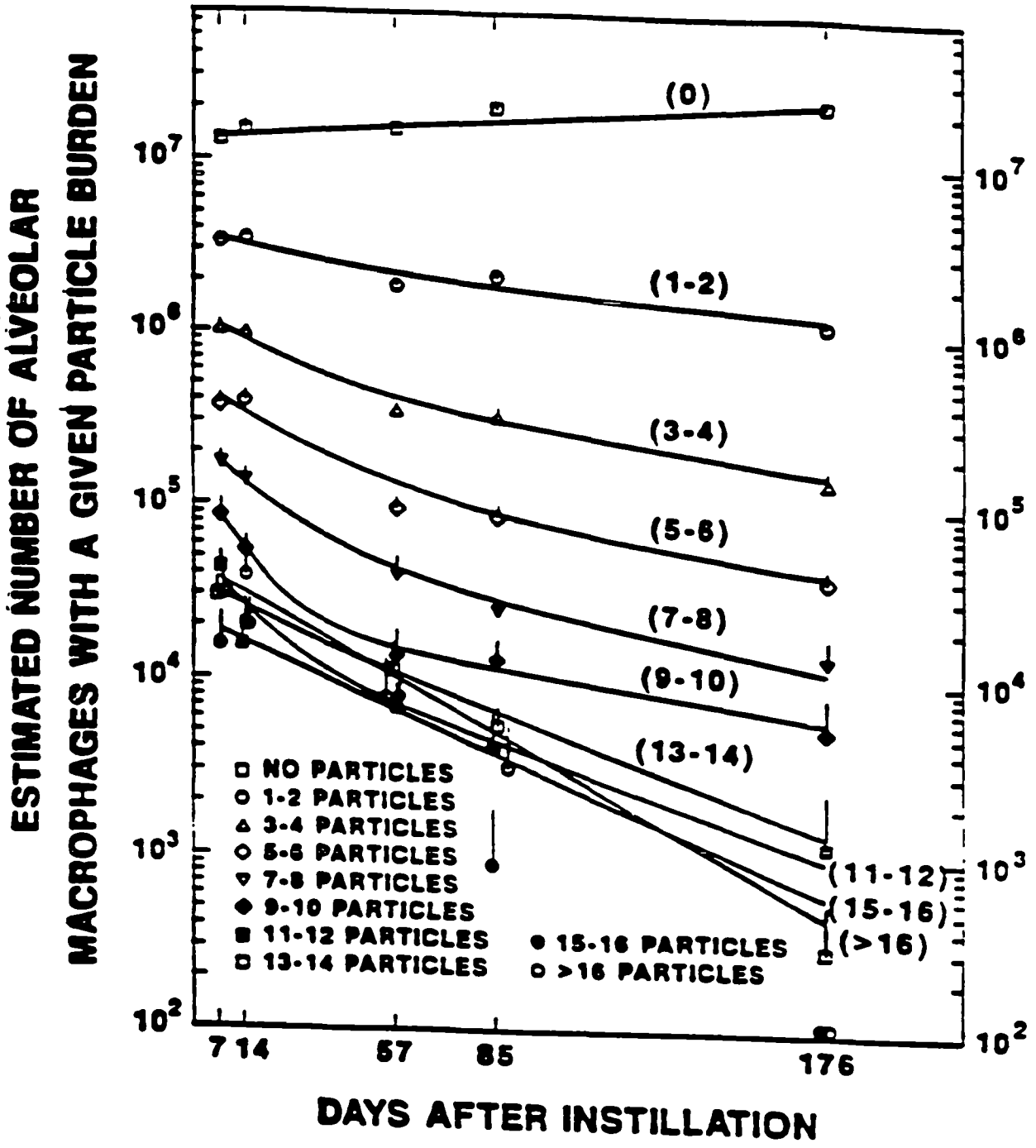

Figure 8-4: Estimated number of AM with an indicated particle load of microspheres over a 174 d period following the instillation of 1.6×10^7 microspheres.

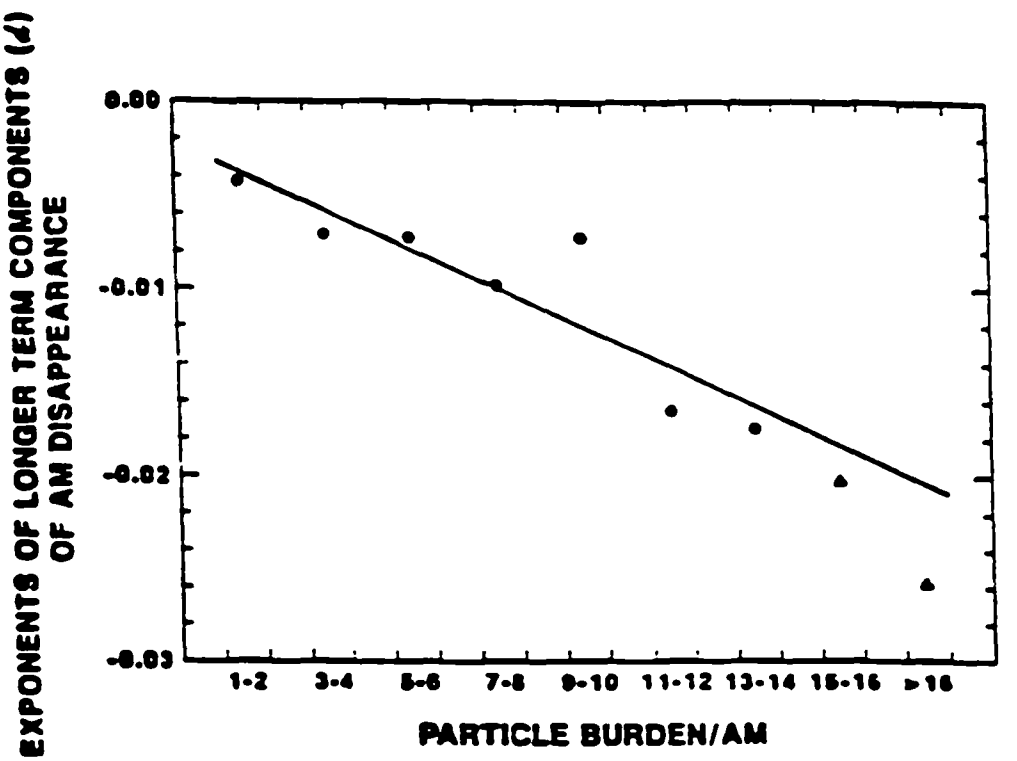

Figure 8-5: Relationship between the slope (exponents) of later phase AM disappearance relative to the particle loads in the AM.

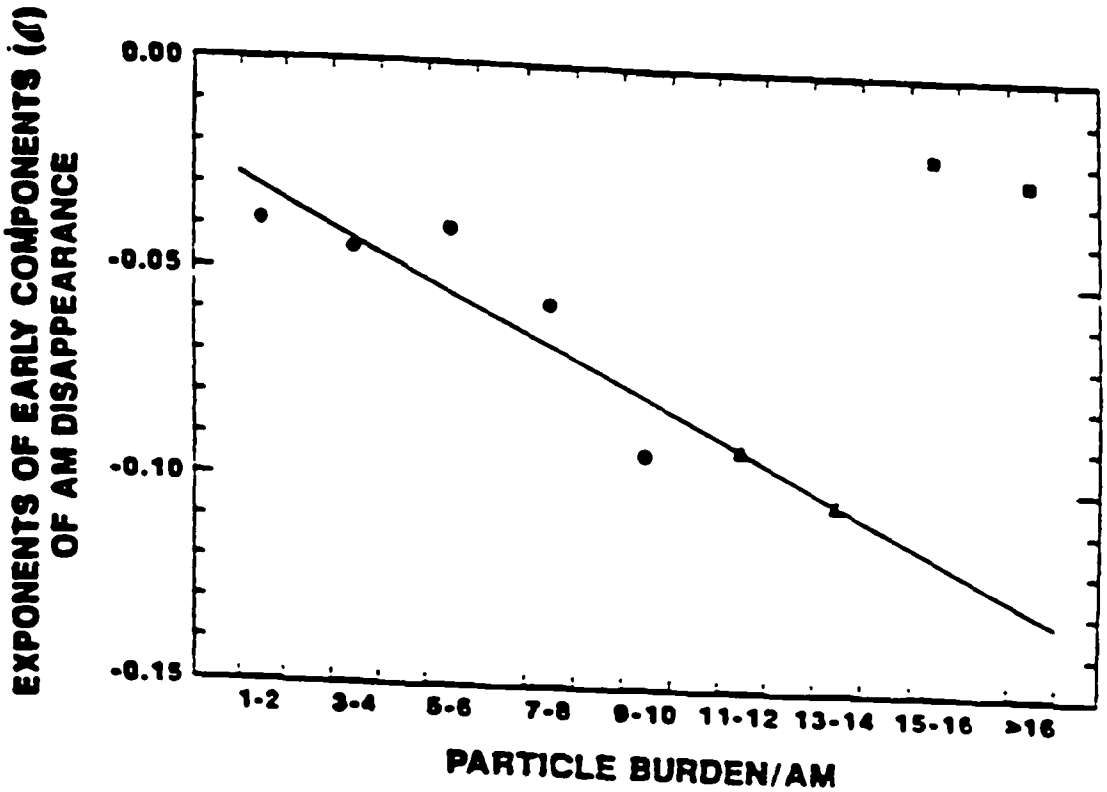

Figure 8-6: Relationship of the rates (exponents) of early component disappearance of AM relative to particle loads in the AM.

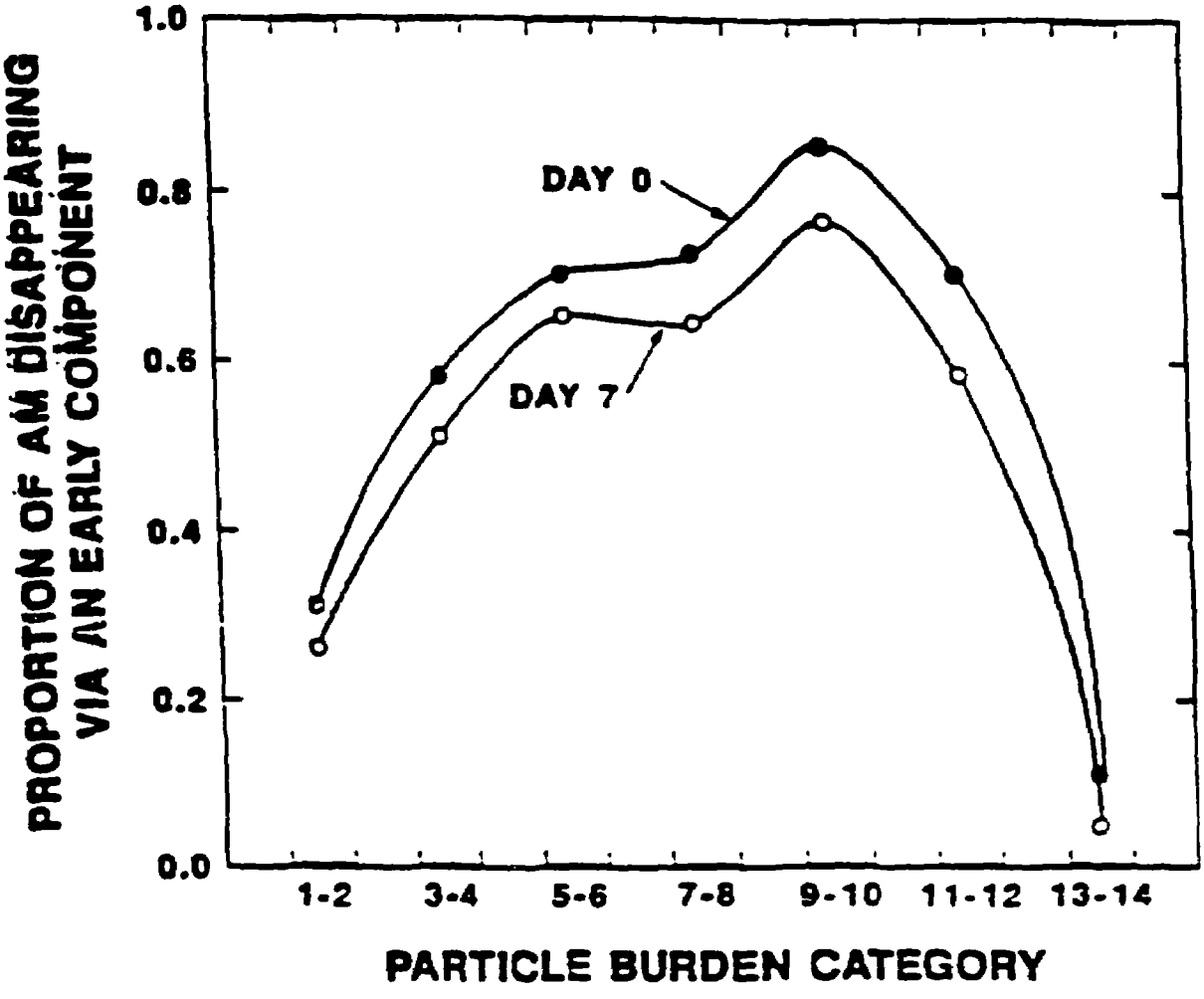

Figure 8-7: Proportions of AM with a given load of particles that disappeared from the total AM population by the early disappearance components as of a theoretical day 0 postinstillation time and as of day 7 after the particles were administered.

disappearance of AM from the various particle categories as observed in the low lung burden component of the above study may not exclusively reflect the translocation of AM from the lung. The observations are also consistent with a gradual redistribution of retained particles among the lung's AM concurrent with the removal of particle-containing AM via the conducting airways *per se*. Consistent with a gradual release of previously endocytosed particles, Lehnert and Morrow (1985) found that a small percentage of a low lung burden of relatively insoluble iron oxide was not associated with lavaged, intact lung free cells over the course of alveolar phase clearance. Potential mechanisms for such a redistribution of retained particles among the AM include: (1) the *in situ* division of particle-containing AM and allocations of the parent AM's particles to daughter cells, (2) the *in situ* autolysis of particle-containing AM and the uptake of the particles by other AM with pre-existing or no particle burdens, (3) the exocytosis of particles by AM and the subsequent phagocytosis of the particles by other AM, (4) the phagocytosis of effete particle-containing AM by other AM, and/or, perhaps, 5) the direct transfer of particles from one AM to another. Experimental support suggestive of at least some of these potential processes comes from a variety of investigations involving AM and other endocytic cell types (Heppleston and Young, 1974; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978). Regardless, an outcome of the apparent differing disappearance rates of AM from the particle categories shown in Figure 8-4 resulted in a relative redistribution in the fractions of the retained lung burdens in the various AM-particle categories over time (Figure 8-8). For example, only ~33 percent of the lung burden of microspheres was in AM that contained 1 to 2 particles as of 7 d after they were instilled, whereas by post-instillation day 176, this particle category accounted for ~65 percent of the retained particles. Nevertheless, if the differing disappearance rates for AM as a function of particle load is due at least in part to particle burden-dependent differences in translocation rates of the AM out of the respiratory tract, the following findings from the above investigation may point to an underlying basis for a slowing of lung clearance at high particle burdens in the lung: (1) as AM reach a given load of particles, a diminishing fraction

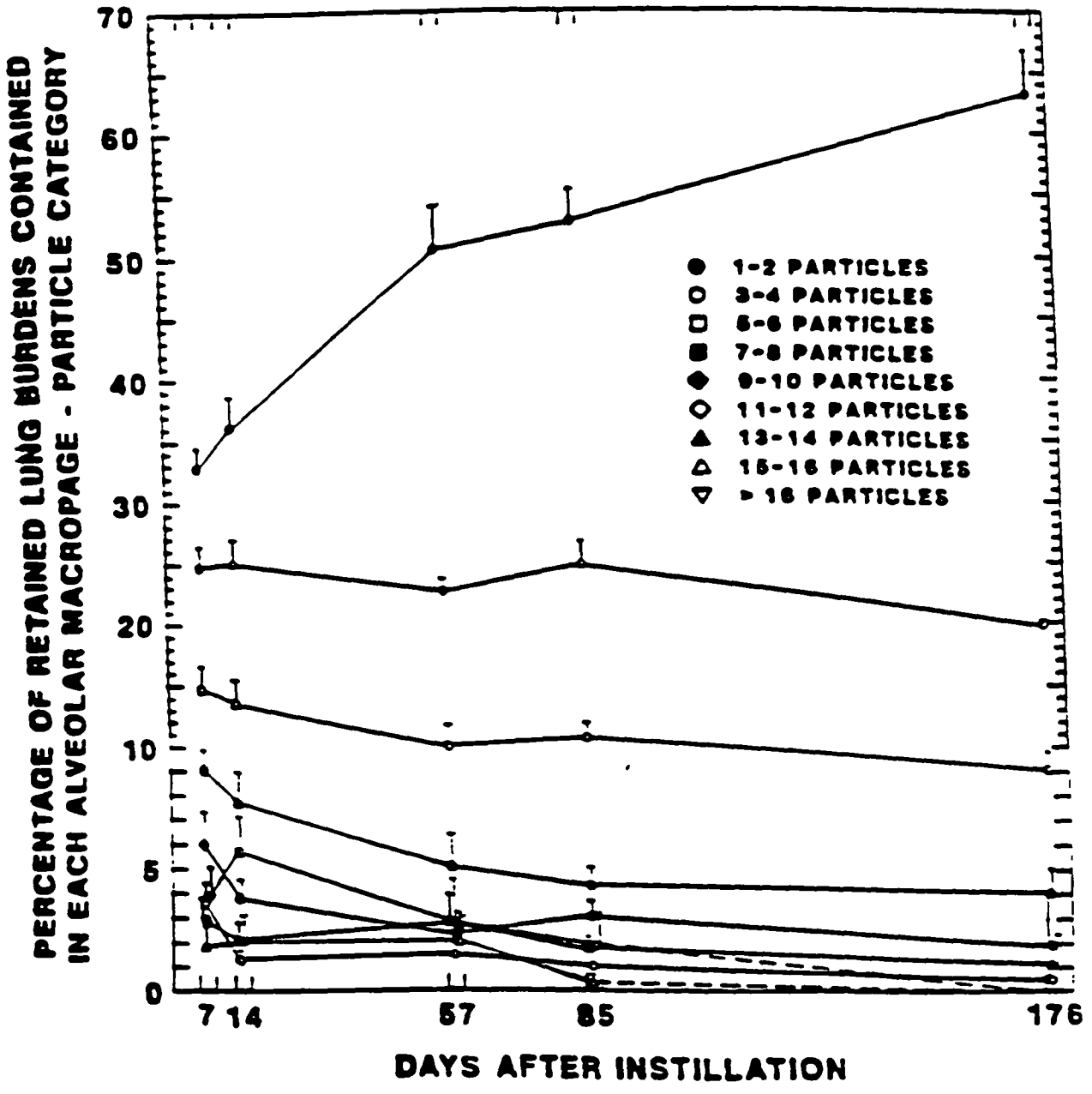

Figure 8-8: Percentage distribution of the retained lung burden in the various particle-AM categories over the course of lung clearance of the 1.6×10^7 microsphere initial burden.

of them is removed via an early component, and (2) AM with high particle loads disappear from the lung essentially by a slower, long-term component.

Similar patterns of AM disappearance as a function of particle load were observed following the deposition of the 2.0×10^8 lung burden, Figure 8-9. That is, AM with the higher initial burdens of particles decreased in numbers more rapidly following the deposition of the particles than did AM with lesser particulate loads. Again, over time, increasing fractions of the retained lung burden became progressively contained in AM with relatively lower particulate burdens per cell, Figure 8-10. The possibility that particles are gradually redistributed among the AM during the course of alveolar phase clearance is further supported by apparent increases in the number of AM containing 1 to 4 particles at the end of the study relative to the number of AM with this particle load as of day 7 after the particles were instilled (Figure 8-9). In the case of the particle overload condition caused by the instillation of the 6.8×10^8 microsphere burden, AM numbers in the higher particle categories showed little change over the course of the 168-d postinstillation period, suggesting that the translocation of AM with the heaviest cellular burdens of particles (i.e., $>\sim100$ microspheres per AM) out of the respiratory tract via the tracheobronchial route was compromised (Figure 8-11). This observation could be related to aggregates of particle-laden AM in the alveoli (Figure 8-12) which, in turn, could contribute to diminished lung clearance of the microspheres, and represent, at least in part, the particle "sequestration" compartment described by other investigators following the deposition of high lung burdens of diesel and other types of particles. Over time, greater percentages of the retained lung burden were contained in AM with the highest particle burdens (Figure 8-13). Further evidence of a gradual release of particles from AM during the course of diminished lung clearance was again observed by increases in AM numbers in the lowest particle category over the study period (Figure 8-9) and the appearance of particle-containing polymorphonuclear leukocytes (Lehnert et al., 1989) and blood monocyte interstitial macrophage-like cells among the lavaged free cell populations (Figure 8-14).

Although lung burdens in the above study were achieved by intratracheal instillations and not by chronic aerosol exposures scenarios, which have led to tumor formation

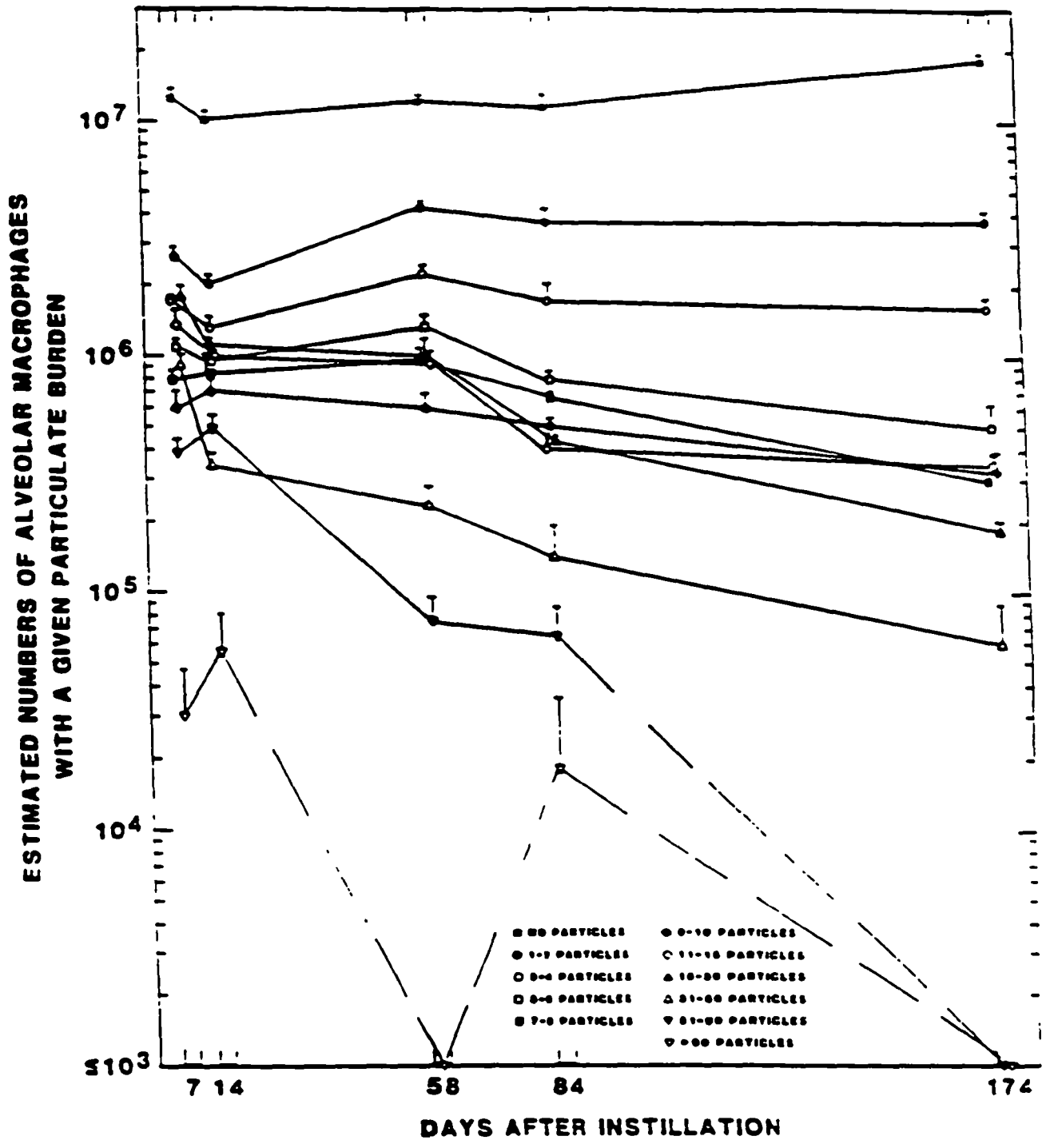

Figure 8-9: Estimated numbers of AM with a given particle burden over the course of clearance of 2.0×10^8 microspheres.

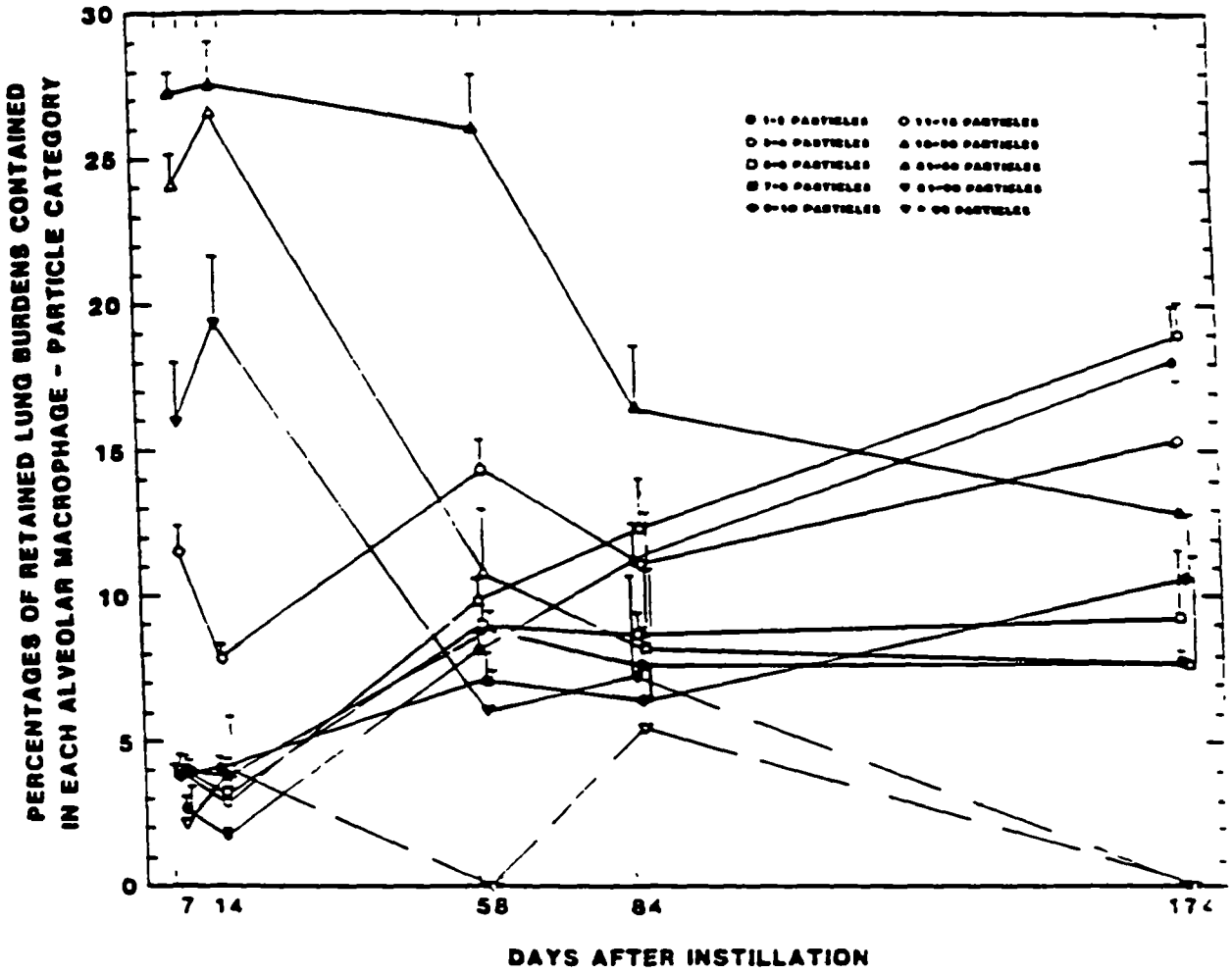

Figure 8-10: Percentage distributions of the retained lung burdens in the various particle-AM categories during the clearance of the 2.0×10^8 initial lung burden of microspheres.

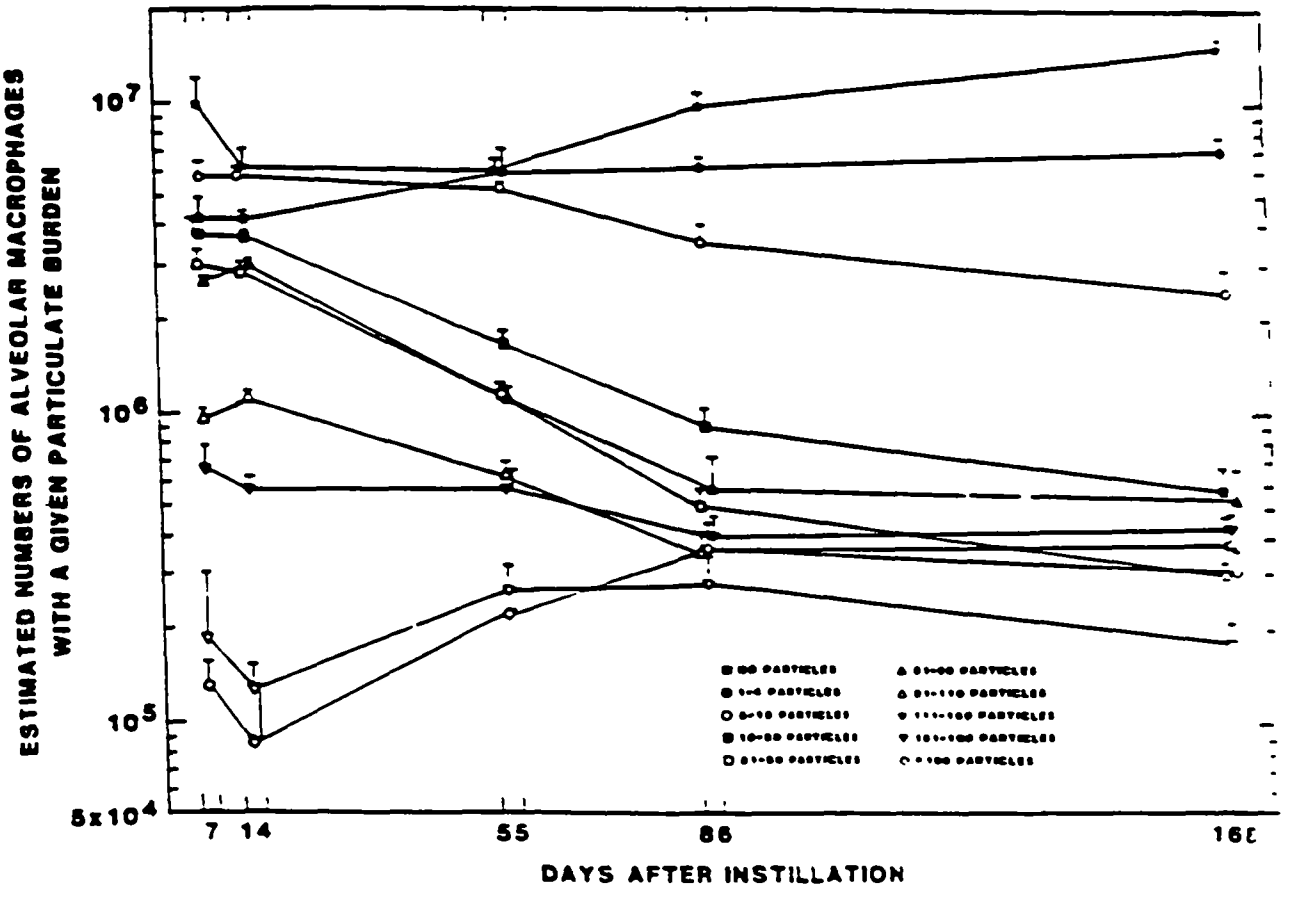

Figure 8-11: Estimated numbers of AM in the indicated particle-containing categories following the deposition of 6.8×10^8 microspheres into the rat's lung.

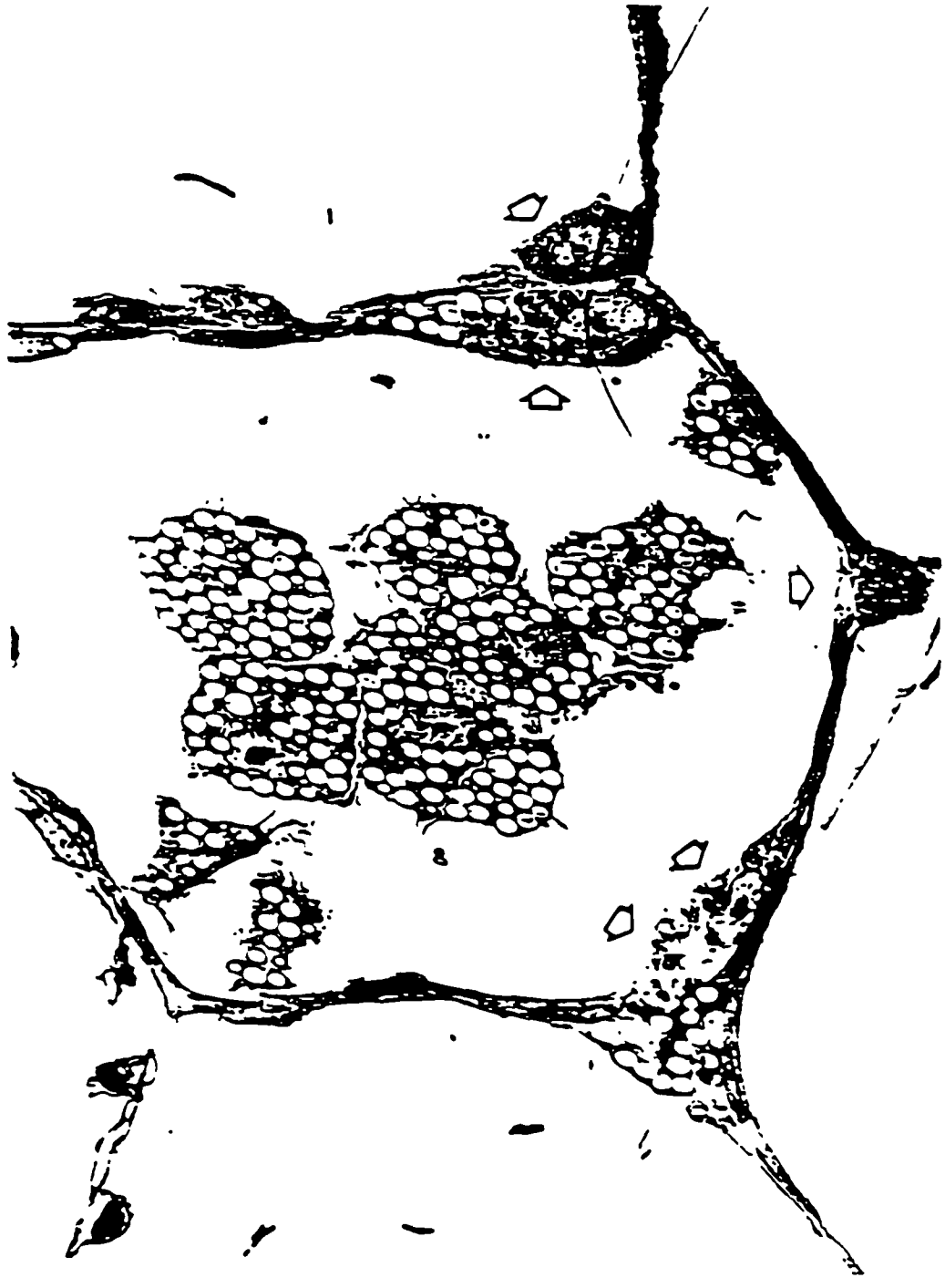

Figure 8-12: Electron micrograph of an aggregate of particle filled AM in an alveolus on day 106 following the deposition of 6.8×10^8 microspheres. Arrows point to Type II pneumocytes, which appear to be present in greater than normal abundance. The microspheres are also found in Type I epithelial cells.

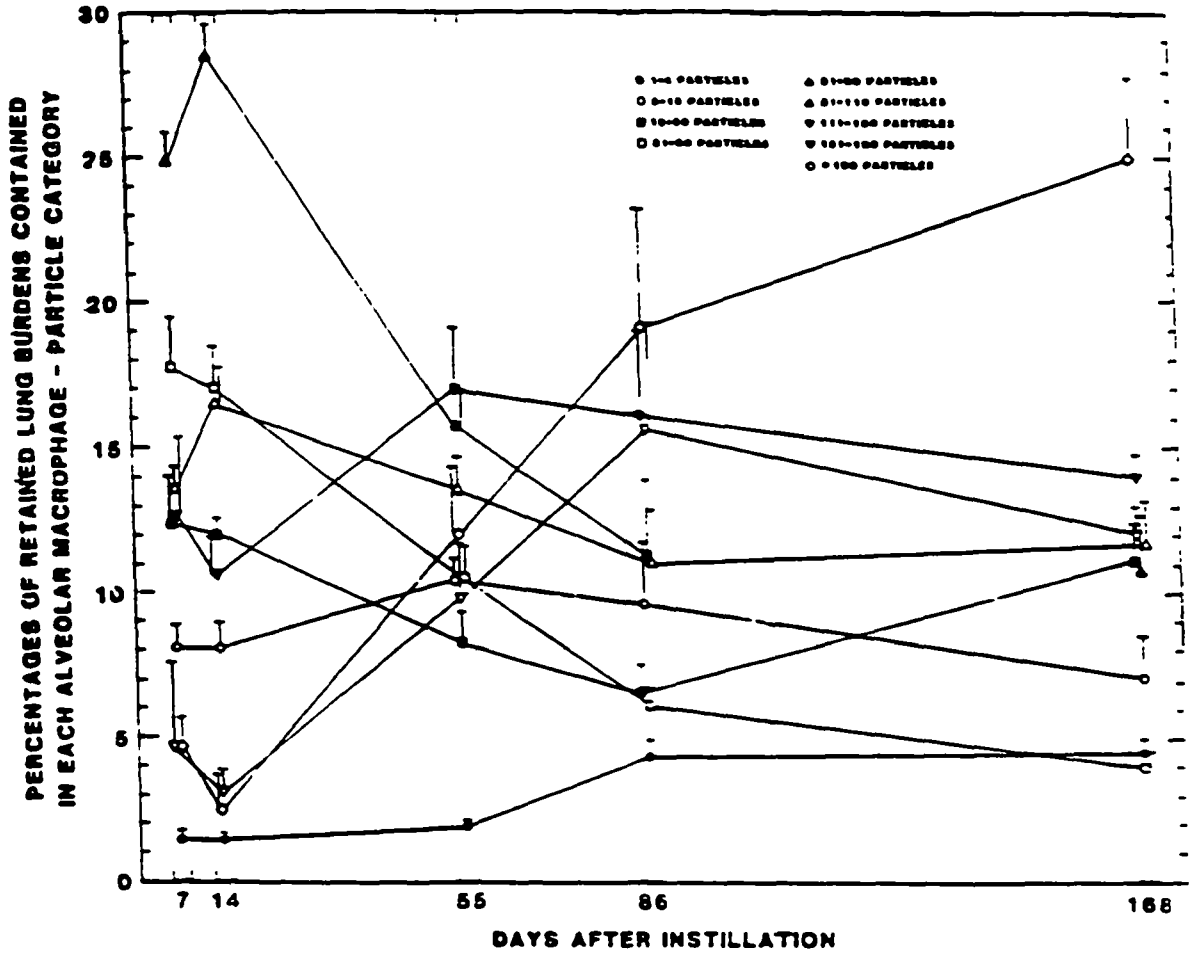

Figure 8-13: Percentages of the retained lung burden contained in the various particle-AM categories following the deposition of 6.8×10^8 microspheres.

in response to a variety of types of materials, the general outcomes may, in principle, be applicable to lung clearance mechanisms at play in the latter condition. If so, two mechanisms that may play major roles in lung retention kinetics following the deposition of aerosolized particles are: (1) the removal of AM from the respiratory tract as a particle-dependent process and (2) the gradual redistribution of particles among the lung's AM population. Factors that may contribute to the phenomenen of particle release by AM, including particle size, shape, composition, and surface features, have not been delineated. Additionally, it remains obscure as to how the magnitude of particulate burdens in AM may influence the particle redistribution process. Regardless, a lack in the fidelity between engulfed particles and AM that initially phagocytized the particles suggests that particles released by AM could be available for interactions with other extra-AM lung cells prior to their endocytosis. Of relevance, the *in situ* disintegration of AM as one source of particle release in the alveoli has been observed following exposure to particulate diesel exhaust (White and Garg, 1981). Experimental information about factors underlying particle release by AM, the kinetics of the release process, and the kinetics and efficiency of particle re-uptake by phagocytes and other lung cells, *inter alia*, is required to establish the cumulative fraction of a lung burden that is available to interact directly with other lung cells over time. Such information would be of use for cellular (epithelial cells) dosimetry estimates while furthering the current understanding of particle-AM interactions that may contribute to the phenomenen of particle overload in the lung.

8.4.2.2. Translocations of Particles to Extra-Alveolar Macrophage Compartment Sites

While the phagocytosis of particles by lung free cells and the mucociliary clearance of the cells with their particulate burdens represent the most prominent mechanisms that govern the fate of particles that deposit in the alveoli, other mechanisms exist that can impact both on the retention characteristics of relatively insoluble particles in the lung as well as on lung clearance pathways for the particles. One mechanism is the endocytosis

Figure 8-14: Micrograph of cells lavaged from a lung 86 d after the deposition of 6.8×10^8 microspheres.

of particles by the Type I pneumocytes (Sorokin and Brain, 1975; Adamson and Bowden, 1978; Adamson and Bowden, 1981) that normally cover >90 percent of the surface area of the alveoli in the lungs of a variety of mammalian species (Crapo et al., 1983). This process may be related to the size of particles that deposit in the lungs and the numbers of particles that are deposited. Adamson and Bowden (1981) found with increasing loads of carbon particles (0.03 mm) instilled into the lungs of mice, more free particles were observed in the alveoli within a few d thereafter; the relative abundance of particles endocytosed by Type I epithelial cells also increased with increasing lung burdens of the particles. These same investigators demonstrated that instilled latex particles with a diameter of 0.1 mm also were endocytosed by Type I pneumocytes when delivered at high lung burdens (4 mg), but latex microspheres with a diameter of 1.0 mm (4 mg) were rarely observed to be engulfed by the alveolar epithelial cells. Although the endocytic ability of Type I pneumocytes must have an as yet to be determined upper particle size limitation, the infrequent appearance of the 1.0 mm spheres in the Type I cells may have been primarily related to the numbers of particles instilled and not necessarily to their size. A 4 mg burden of 0.1 mm diam latex particles is equivalent to 8×10^{12} particles whereas a 4 mg burden of 1.0 mm diam latex particles is composed of 8×10^{9} particles. Regardless, diesel particles with volume median diameters between 0.05 and 0.3 mm (Frey and Corn, 1967; Kittleson et al., 1978) would be expected to be within the size range for engulfment by Type I cells should suitable encounters occur. Indeed, it has been demonstrated that diesel particles are endocytosed by Type I pneumocytes *in vivo* (White and Garg, 1981). Adamson and Bowden (1981) postulated that when acutely administered high lung burdens of particles exceed the lungs capacity to recruit for additional phagocytes into the alveolar space compartment to phagocytize the delivered burden, the presence of free particles increases the likelihood of particle encounters with Type I cells. A hypothetical extension of this postulate is: some fraction of particles which are deposited in the alveoli gain entry into Type I epithelial cells even when deposited lung burdens are low or when the mechanism of particle phagocytosis by AM is not overwhelmed. Alternatively, the process of particle phagocytosis (i.e., particle encounters and phagocytosis), by AM seemingly would have to be kinetically accomplished with 100 percent efficiency within a postdeposition time span that would preclude particle

uptake by the Type I cells. Unfortunately, information on the kinetics of particle endocytosis by Type I cells relative to that by AM is scant. However, the above extension of the Adamson and Bowden postulate is consistent with some existing observations. For example, even when relatively low burdens of particulate agents are deposited in the lungs, some fraction of the particles usually appear in the regional lymph nodes (Ferin and Feldstein, 1978; Lehnert, 1989); as will be discussed, endocytosis of particles by Type I pneumocytes is an initial, early step involved in the passage of particles to the lymph nodes. Assuming particle phagocytosis is not sufficiently rapid or perfectly efficient, increasing numbers of particles would be expected to gain entry into the Type I epithelial cell compartment during chronic aerosol exposures. Additionally, if particles are released on a continual basis by alveolar phagocytes that initially sequestered them after lung deposition, some fraction of the "free" particles so released could also undergo passage from the alveolar space into the Type I cells.

As previously indicated, the endocytosis of particles by Type I pneumocytes represents only the initial stage of a process that can lead to the accumulation of particles in the lung's interstitial compartment and the subsequent translocation of particles to the regional lymph nodes. As shown by Adamson and Bowden (1981), a vesicular transport mechanism in the Type I pneumocyte can transfer particles from the air surface of the alveolar epithelium into the lung's interstitium where particles may be phagocytized by interstitial macrophages or they may remain in a "free" state for a poorly defined period of time that may be dependent on the physicochemical characteristics of the particle. The lung's interstitial compartment, accordingly, represents an anatomical site for the retention of particles in the lung. Whether or not AM and perhaps PMN that have gained access to the alveolar space compartment and phagocytize particles there also contribute to the particle translocation process into the lung's interstitium remains a controversial issue. However, it is widely believed that once AM, at least, assume occupancy in the alveoli, they do not reenter the lung's interstitium (Roser, 1970; Adamson and Bowden, 1978; Brain et al., 1977). Adamson and Bowden (1978, 1981), for example, found no ultrastructural evidence that AM migrate into the lungs intersitium after the acute deposition of a heavy burden of carbon or of latex microspheres into the lungs of mice. Likewise, Lauweryns and Baert (1977) also found no morphologic

evidence for an AM migration through the alveolar epithelium after intratracheal instillations of ferritin or carbon. On the other hand, Corry and co-workers (1984) intratracheally instilled radiolabeled AM into syngeneic guinea pigs and found radioactivity in the hilar lymph nodes as well as the presence of radiolabeled cells in autoradiographic preparations made from nodal cell suspensions obtained over a 24 to 72 h period after the instillations. Lehnert and co-workers (1986) also examined mononuclear phagocytes from the tracheobronchial lymph nodes of rats for the presence of particles after the intratracheal instillation of polystyrene microspheres. These investigators found a disconcordance between the frequency distributions of the microspheres in the nodal macrophages over a 30 d period after the particles were administered relative to the particle distributions in lavaged AM (i.e., the nodal phagocytes contained lesser particle cellular burdens than did the AM). These latter findings suggest that if AM do translocate across the alveolar epithelial barrier, the process may be limited by the magnitude of their particulate burdens. These collective findings also suggest that at least some, if not most, of the macrophages observed to contain particles in the lungs interstitium after particle deposition represent resident interstitial and perhaps newly recruited blood monocytes that have phagocytized particles translocated across the alveolar epithelial barrier (Adamson and Bowden, 1978; Bowden and Adamson, 1984). It should be pointed out, however, that the migration of AM into the interstitium may be species dependent. Evidence that such migration of AM may importantly contribute to the passage of particles to the interstitial compartment and also be involved in the subsequent translocation of particles to draining lymph nodes has been obtained with the dog model (Harmsen et al., 1985).

The fate of particles once they enter the lung's interstitial spaces remains unclear. Some particles, as previously indicated, are phagocytized by interstitial macrophages whereas others apparently can remain in a free state in the interstitium for some time without being engulfed by interstitial phagocytes. It is currently unknown what fraction of the interstitial macrophages may subsequently enter the alveoli with their engulfed burdens of particles and thereby contribute to the size of the resident AM population over the course of lung clearance. Moreover, no investigations have been conducted to date to assess the influence

that an interstitial macrophage's burden of particles may have on its ability to migrate into the alveolar space compartment.

It appears evident that at least some particles that gain entry into the interstitial compartment can further translocate to the extrapulmonary regional lymph nodes. This process apparently can involve the passage of free particles as well as particle containing cells via lymphatic channels in the lungs (Harmsen et al., 1985; Ferin and Feldstein, 1978; Lee et al., 1985). It is conceivable that the mobility of the interstitial macrophages could be particle burden limited, and under conditions of high cellular burdens, a greater fraction of particles that accumulate in the lymph nodes under conditions of high lung burdens may reach these sites as free particles. Whatever the process, existing evidence indicates that when lung burdens of particles result in the particle overload condition, particles accumulate both more rapidly and abundantly in lymph nodes that receive lymphatic drainage from the lung (Ferin and Feldstein, 1978; Lee et al., 1985).

8.4.2.3. Some Potential Mechanisms that May Underlie the Appearance of an Alveolar Macrophage "Sequestration" Compartment for Particles During Particle Overload

Animal studies have revealed that impairment of alveolar clearance can occur following chronic exposure to particulate diesel exhaust (Griffis et al., 1983; Wolff et al., 1987; Vostal, 1982; Lee et al., 1983). Several other experimental animal studies involving chronic aerosol exposures to a variety of other diverse materials have also demonstrated that the lung retention of particles can be increased following the deposition of excessive lung burdens of particles (Lee et al., 1986; Lee et al., 1988; Ferin and Feldstein, 1978). In that high lung burdens of different types of particles result in diminutions of normal lung clearance kinetics, or result in what is now called particle overloading, this outcome appears to be more related to the mass and/or volume of particles in the lung than to the nature of the particles *per se*. Regardless, as pointed out by Morrow (1988), particle overloading in the lung modifies the dosimetry for particles in the lung and thereby can alter toxicologic responses to the particles. Unfortunately, information on particle burden and particle

overload relationships in the human lung are not available as with experimental animal models.

The mechanisms that underlie particle-load dependent retardations in the rate of removal of particles from the lung and the corresponding functional appearance of an abnormally slow clearing compartment, or particle "sequestration" compartment, are likely numerous. As previously discussed, one potential site for particle sequestration is the extra-macrophagic containment of particles in the lung's Type I epithelial cells. Information on the retention kinetics for particles in the Type I epithelial cells is nonexistent, and no information as to how the vesicular transport of particles across the Type I cell may be exhausted or otherwise modified during particle overload is currently available. Also, no morphometric analyses have been performed to date to estimate what fraction of a retained lung burden may be contained in the lung's Type I pneumocyte population during the condition of lung overloading with particulate diesel exhaust or other types of particles.

Another anatomical region in the lung that may be a slowly clearing site is the lung's interstitial compartment. Little is known about the kinetics of removal of "free" particles or particle-containing macrophages from the lung's interstitial spaces or what fraction of a retained burden of particles is contained in the lung's interstitium during particle overload. The gradual accumulation of particles in the regional lymph nodes and the appearance of particles and cells with associated particles in lymphatic channels and in peribronchial and perivascular lymphoid tissue (Lee et al., 1985; White and Garg, 1981) suggest that the mobilization of particles from interstitial sites via local lymphatics is a continual process.

Indeed, it is clear from histologic observations of the lungs of animals chronically exposed to diesel particles that Type I pneumocytes, the interstitium, the lymphatic channels, and pulmonary lymphoid tissues are sites that could represent subcompartments of a more generalized particle sequestration compartment. Strom (1984) has reported that lesser percentages of retained lung burdens of diesel particles are lavageable from lungs of animals chronically exposed to diesel particles relative to those percentages of the retained lung burdens that are lavageable following acute exposures. The basis for this observation may, at least in part, be related to the relative fraction of a chronically administered burden of particulate diesel that is contained in extra-AM sites that are not susceptible to lavage.

Although the above mentioned sites must be considered as potential contributors to the diminutions of lung clearance during particle overload, disturbances in particle associated AM-mediated clearance is undoubtedly the predominant cause inasmuch as the AM are the primary reservoirs of containment of deposited particles. The mechanism underlying a failure of AM to translocate from the alveolar space compartment in lungs with high particulate burdens remains obscure, although a hypothesis concerning the process have recently been offered. Morrow (1988) has proposed that the condition of dust overloading in the lungs is caused by a loss in the mobility of particle engorged AM and that such an impediment is related to the cumulative volumetric load of particles in the AM. Thus, according to this hypothesis, the essentially continuous phagocytosis of particles by AM during chronic exposures to high mass concentrations of particles gradually results in a volume load in the AM that in some manner results in their immobilization. Morrow (1988) has further estimated that the clearance of an AM is impaired when the particulate burden in the AM is of a volumetric size equivalent to ~60 percent of the normal volume of the AM. Hence, a primary determinate of an incapacitation of AM-mediated particle clearance may not be due to cumulatively retained lung mass burden, but rather, the emergence of the particle overload condition and the extent of diminished lung clearance may more directly relate to the volumetric load of a retained burden in the lung and its distribution among members of the lung's AM population. Some recent evidence is consistent with the hypothesis that an AM's volume load of particles can decreased the removal of AM from the alveoli. Specifically, Oberdorster and co-workers (1988) assessed the alveolar clearance of smaller (3.3 mm dia.) and larger (10.3 mm dia.) polystyrene particles, the latter ofwhich are volumetrically equivalent to ~60 percent of the average normal volume of an AM, after they were intratracheally instilled into the lungs of rats. Whereas both sizes of particles were found to be phagocytized by AM as of one d after deposition, only minimal lung clearance of the larger particles was observed over an ~200-d postinstillation period.

Other processes may also be involved in preventing AM from leaving the alveolar compartment with their phagocytized particulate burdens under conditions of particle overload in the lung. Clusters or aggregates of particle-laden AM in the alveoli are typically found in the lungs of experimental animals that have received high lung burdens of a variety

of types of particles (Lee et al., 1985), including particulate diesel exhaust (White and Garg, 1981; McClellan et al., 1982), see Figure 8-14. The aggregation of AM may represent a major, if not the primary explanation for the reduced clearance of particle-laden AM during particle overload. The mechanism(s) responsible for the agglutination of the AM has not been elucidated to date. Conceivably, the clustering of AM may be related to the Type II hyperplasia observed in lungs that have received high lung burdens of particle and an abnormal abundance of Type II cell-derived products, phospholipids, in the alveoli; after short term exposure to diesel exhaust, at least, lavageable phospholipid has been found to increase (Wright, 1986). Support for this possibility comes from a study by Ferin (1982) in which the lung clearance of TiO_2 was shown to cease when a condition of phospholipidosis was experimentally induced. Also, the appearance of large "foam" cells that are observed following the deposition of high lung burdens of a variety of particle types is consistent with this possibility. Another underlying factor that may be involved in the clustering effect is a continual alteration in the permeability of the alveolar epithelial barrier under conditions of particle overload (Creutzenberg et al., 1989). In this case, "sticky" factors derived from the blood compartment, such as fibronectin, could contribute to AM adhering to one another. Still another potential mechanism for the agglutination of AM could be related to cytokines released from lymphocytes (Garcia-Moreno and Myrvik, 1977). Some evidence, however, suggests that lymphokines (e.g., macrophage migration inhibition factor) may not be the dominant mechanism responsible for AM aggregation in the air spaces. While lung lymphocytes do increase following diesel exposure (Strom, 1984), AM aggregation following TiO_2 deposition in the lung occurs with the general absence of excessive lung lymphocytes (Lee et al., 1986). Whatever the underlying mechanism(s) for the AM agglutinating response, it is worthy to note that AM lavaged from the lungs of diesel exhaust-exposed animals continue to demonstrate a propensity to aggregate (Strom, 1984). This observation suggests that the surface characteristics of the AM are fundamentally altered in a manner that promotes their adherence to one another in the alveolar region and that AM aggregation may not simply be due directly to their abundant accumulation as a result of a particulate volumetric load immobilization.

8.4.3. Summary: Pulmonary Clearance of Diesel Exhaust Particulate Matter

The animal studies examined indicate that exposure to diesel exhaust unequivocally increases lung particle burden in a dose-dependent fashion with high exposure levels (3.5 to 7.0 mg/m^3) resulting in compromised clearance of particulate matter. An initial, relatively rapid clearance phase is mediated by mucociliary transport. This process is especially important for removal of particles from the major airways and terminal bronchioles. A slower clearance phase involving aggregates of alveolar macrophages is very important in the pulmonary clearance of particulate matter. The role of alveolar macrophage activity and the potential for overloading of this function are of concern relative to removal of particles from the lung. The development of lung tumors in experimental laboratory animals following chronic exposures to particulate diesel exhaust occurs under conditions in which alveolar macrophage-mediated particle clearance from the lung is compromised. Tumors have also been found to develop with other types of particles when this clearance mechanism is diminished. Thus, reductions in the functional activity of the lung's alveolar macrophage population in the clearance process appears to be intimately related to the carcinogenic response to high lung burdens of particles, generally. Findings that tumors develop in the lungs of laboratory rats at lesser lung mass or volume burdens of diesel particles than with a substance like titanium dioxide suggest that the carcinogenic response, however, is not exclusively related to an over abundance of particles in the lungs *per se*. Most likely, the organic components on diesel particles, many of which have demonstrated carcinogenic activity, are importantly involved in the development of lung tumors. The lung's pulmonary macrophages, which phagocytize deposited diesel particles, probably participate in the gradual *in situ* extraction and metabolism of procarcinogens on diesel particles. The extraction of potentially carcinogenic compounds from particles is addressed more fully in the following scetion. Additionally, the normal tumoricidal activities of the pulmonary macrophages may be compromised upon interaction with excessive numbers of diesel particles, and diesel particle-macrophage interactions could lead to the generation of reactive oxygen species that have been shown to be at least mutagenic. Caution must be exercised in extrapolating observations made in animal models to the human condition. Processes and

potential mechanisms discussed herein have largely been derived from animal data. Further research is required to determine how the activities of human pulmonary macrophages in response to particulate diesel exhaust compare with pulmonary macrophages from experimental animals. Most importantly, valid dosimetry for the human condition requires the elucidation of the underlying mechanistic bases involved in the development of lung tumors following chronic particulate diesel exhaust exposure.

The extrapolation of the reported pulmonary retention and clearance data to humans appears to be tenuous at present due to the high exposure levels used in the animal studies, and interspecific variability in physiological function and anatomy of the respiratory tract which may alter the threshold for clearance overloading. Additionally, humans are exposed to much lower particle concentrations, and thus not likely to exhibit impaired clearance. Partially offsetting the impaired clearance during lung overloading in rats is the normally greater long-term clearance times for humans. Bailey et al. (1982) reported $t_{1/2}$ values of nearly 1 yr.

8.5. DESORPTION OF CONSTITUENTS FROM DIESEL EXHAUST PARTICLES

8.5.1. Bioavailability of Agents Adsorbed to Diesel Exhaust Particles

Bioavailability of toxic organics in diesel exhaust appears to be dependent on the dissociation of these compounds from diesel particles (Vostal, 1983) and on the persistence of the particulate matter which is a function of deposition and pulmonary clearance. That toxicity of the organic compounds is increased by the presence of particles has been demonstrated for benzo[a]pyrene (Saffiotti et al., 1964; Creasia and Nettesheim, 1974; Henry et al., 1975). These studies indicated that a higher incidence of lung carcinomas was observed when B[a]P was adsorbed onto iron oxide particles than when intratracheally instilled in pure form. However, the mechanism of this greater toxicity has not been elucidated. Several studies have provided evidence that polycyclic aromatic hydrocarbons (PAHs) including 1-nitropyrene and benzo[a]pyrene may be released from diesel exhaust

particles, thus demonstrating the potential bioavailability of carcinogenic agents from diesel exhaust. Pepelko and Peirano (1983) in summarizing U.S. EPA studies on health effects of diesel engine emissions described a study designed to assess the effect of inhaled diesel exhaust upon the bioavailability, distribution, metabolism, and excretion of intratracheal instilled B[a]P. Results of this phase of the study indicated that exposure to diesel exhaust had no significant effect on the uptake or distribution of the administered B[a]P. More recent studies, described below, have also studied the relationship between the particle phase of diesel exhaust and the bioavailability of the associated organics.

Sun et al. (1984) provided additional information comparing the disposition of particle-adsorbed B[a]P (0.1 percent by weight) and pure B[a]P following 30 min nose-only inhalation by F344 rats. Long-term lung retention (percentage retained after 7 d) of particle-adsorbed ^{3}H-B[a]P was approximately 230-fold greater than that for pure ^{3}H-B[a]P. Pulmonary clearance of particle-associated ^{3}H was biphasic with an initial $t_{1/2}$ of 1 h and a second phase $t_{1/2}$ of 18 d, the latter representing clearance of 50 percent of the initially deposited radiolabel. Clearance of pure B[a]P aerosol was >99 percent within 2 h and was apparently due to pulmonary and mucous membrane absorption into the blood rather than by mucociliary clearance and subsequent ingestion (Sun et al., 1982). Of the radiolabel retained in the lungs, 65-76 percent was B[a]P, 13 to 17 percent was B[a]P-phenol, and 5 to 18 percent was B[a]P-quinone. Tissue distribution and excretion of ^{3}H were similar to previous studies (Sun et al., 1982) where B[a]P was adsorbed onto gallium oxide (Ga_2O_3) particles, thus suggesting that adsorption to the carbonaceous diesel exhaust particles prolongs the release of B[a]P.

Although the Sun et al. (1984) study demonstrated the bioavailability and subsequent biotransformation of B[a]P to several metabolites, the epoxide intermediates known to be carcinogenic (Sims et al., 1974; Slaga et al., 1976) were not identified. However, B[a]P-phenol metabolites are reported to be mutagenic (Glatt and Oesch, 1976; Wislocki et al., 1976; Wood et al., 1976).

A companion study (Bond et al., 1986) examined the biological fate of ^{14}C-1-nitropyrene (^{14}C-NP) both in pure form and adsorbed to diesel exhaust particles following 1 h nose-only inhalation by male F344 rats. Concentrations of ^{14}C-NP ranged from 50 to

1100 ng/L air, and diesel particle concentrations, where utilized, ranged from 3.7 to 6.1 μg/L air. The results indicated that long-term lung retention of ^{14}C-NP adsorbed onto diesel exhaust particles was 80-fold greater than that for pure ^{14}C-NP. These studies also demonstrated that adsorption onto the diesel exhaust particles prolongs the release of the PAHs.

In these studies retention of ^{14}C-B[a]P and ^{14}C-NP was less than that for diesel particles alone, thus release from the particles was occurring. The contention that adsorption to particles affects the bioavailability of diesel exhaust PAHs is affirmed by these studies.

Bond (1987) summarized the importance of particle association in the clearance of adsorbed organic compounds, and emphasized the necessity for understanding the physical and chemical interactions between the particle and these organic compounds. Also emphasized was the significance of alveolar macrophage activity relative to deep lung clearance of particles and potential for metabolism of the organics to reactive intermediates.

Ball and King (1985) studied the disposition and metabolism of ^{14}C-labeled 1-nitropyrene (>99.9 percent purity) coated onto diesel exhaust particles. A single dose of 1-NP (380 μg/g particle) was intratracheally administered (in 0.2-mL buffered saline) at a particle dose of 5 mg/rat. Another group of rats (number not specified) received the labeled 1-nitropyrene in 0.5 mL intragastrically. Additional groups of AGUS strain rats raised conventionally or germ-free received intraperitoneal injections of ^{14}C-NP to determine the role of gastrointestinal flora on the metabolism of 1-NP.

Regardless of the route of administration, greater than 50 percent of the ^{14}C was excreted within the first 24 h, 20-30 percent appeared in the urine, and 40 to 60 percent was excreted in the feces. Intestinal flora was essential for the formation of the metabolite, 6-hydroxy-N-acetyl-1-aminopyrene, which was mutagenic (with S9) in *Salmonella typhimurium* strain TA98. The intestinal flora were also required for making first-pass biliary metabolites available (by conjugation-hydrolysis reactions) for enterohepatic circulation. The ^{14}C excretion pattern for intratracheally instilled compound was nearly identical to that of orally administered compound. For these animals, 16 to 38 percent of the unexcreted dose was in the gastrointestinal tract and 5 to 8 percent remained in the lungs. Traces of radiolabel

were detected in the trachea and esophagus. Five to 12 percent of the radiolabel in the lung copurified with the protein fraction, thus indicating protein binding of the 1-NP-derived ^{14}C. However, the corresponding DNA fraction contained no ^{14}C above background levels. This study demonstrated that the excretion kinetics and the metabolic profiles for 1-NP are similar for oral and pulmonary routes of administration.

As a means of assessing bioavailability of particle-associated compounds, a comparison of the mutagencity of compounds extracted from diesel exhaust particles by dichloromethane or by biological fluids (serum, lavage fluid) was conducted by Brooks et al. (1981). Diesel exhaust particles were obtained from a 5.7-L engine operated at idle and from a 2.1-L engine operating on the FET cycle. Particles from these sources were extracted using dichloromethane or were subjected to incubation with the biological fluids for 6, 24, 48, 72, or 120 h at 37°C. Mutagenicity was assessed using *S. typhimurium* strains TA98 and TA100 with and without S9. For the diesel exhaust particles incubated with biological fluids, there was no increase in mutagenic activity with increased incubation time. Based on the concentration response curves for the mutagenicity tests, the biological fluids extracted only 3 to 10 percent of the activity extracted by dichloromethane. The authors qualified the findings, however, by indicating that the time frame of the experiments does not reflect that of the long-term pulmonary clearance processes, and that the biological fluids may bind or inactivate the mutagenic compounds *in vitro,* but interactions with cells *in vivo* may still occur. The binding of compounds by biological fluids may not actually inactivate the compounds, but rather allow transport to target cells and subsequent activation. The potential for *in vivo* extraction, detoxification or activation of particle-associated compounds makes predictive use of *in vitro* systems tenuous until additional research combining *in vivo* and *in vitro* tests is conducted.

Similar findings were reported by King et al. (1981). In this study, lung lavage fluid and lung cytosol fluid extracts of diesel exhaust particles were not mutagenic. Serum extracts of diesel particles did exhibit some mutagenic activity but this was considerably less than that for organic solvent extracts. Furthermore, the mutagenic activity of the solvent extract was significantly reduced when combined with serum or lung cytosol fluid suggesting protein binding or biotransformation of the mutagenic components.

The involvement of alveolar and interstitial macrophages in determining the biovailability of adsorbed organics is demonstrated by several studies. As indicated by Lehnert (1988) and Godleski et al. (1988), translocation and phagolysosomal solubilization of particles may be instrumental in affecting pulmonary clearance of the particles and biovailability of the particle-associated constituents.

Bond et al. (1984) provided evidence that alveolar macrophages from beagle dogs metabolized B[a]P in solution as well as B[a]P coated on diesel exhaust particles. Culture media incubations produced B[a]P-9,10-diol and B[a]P-7,8-diol and incubations using the pulmonary macrophages produced B[a]P-7,8-diol and B[a]P-4,5-diol. Both incubation mixtures formed small quantities of B[a]P phenols and quinones. The significance of these findings is the demonstration of formation by pulmonary macrophages of B[a]P metabolites that are proposed proximate carcinogens and that these metabolites are derived from diesel exhaust particle-associated B[a]P. These findings confirmed an earlier report by King et al. (1983) indicating that pulmonary macrophages metabolized mutagenic nitroaromatics found in diesel exhaust particles.

8.5.2. Extraction of Carcinogens from Particles by Alveolar Macrophages and Other Cell Types

High lung burdens of chronically administered particles such as titanium dioxide (TiO_2) have been shown to produce lung tumors in laboratory animals (Lee et al., 1986). Experimental results such as these, as well as other findings (Ishinishi, 1986), suggest that an over abundance of particles in the lung, however innocuous the particle type, can lead to the development of lung tumors. However, some existing evidence suggests that the development of tumors in diesel exhaust-exposed animals (Mauderly et al., 1987) may not be related exclusively to a high lung burden of particles *per se*. Specifically, the lung burdens of TiO_2 that have been associated with the production of lung tumors in laboratory rats (Lee et al., 1986) substantially exceed lung burdens of diesel particles that have been associated with tumor development (Mauderly et al., 1987). Still other data suggests that the quality (e.g., physicochemical characteristics), of a particle, or, in other words, a pure

particle effect may be of primary importance in producing lung tumors. For example, Kawabata et al (1986) found that the intratracheal instillation of repeated doses of carbon black was as effective in producing lung tumors as was the same mass of instilled particulate diesel exhaust. Thus, it remains possible that a particle composed of carbon may be inherently more carcinogenic than a particle with the physicochemical characteristics of a material like TiO_2. Even so, approximately 10 to 20 percent of the particulate mass of diesel particles is extractable by organic solvents (Frey and Corn, 1967). Among the extractable compounds are numerous mutagenic and carcinogenic chemicals; for example, polycyclic aromatic hydrocarbons (PAH) (Barth and Blacker, 1974; Huisingh et al., 1978; Lee et al., 1980). Experimental findings that metabolites of benzo(a)pyrene (B[a]P) and DNA adducts are formed in the lungs of rats following exposure to diesel exhaust and B[a]P adsorbed onto carbon suggest that the PAH in diesel particles may contribute significantly to the carcinogenic response (Wolff et al., 1989). Thus, the extraction of procarcinogenic compounds from diesel particles that deposit in the lung may be one underlying factor in the development of lung tumors associated with the pulmonary deposition of diesel particles. Such a process would potentially provide a means for the bioavailability of cancer producing agents to sensitive target cells. One mechanism by which carcinogenic agents could be eluted from diesel particles is by extraction into extracellular fluids lining the respiratory tract. Siak et al (1980) assessed the mutagenicity of material extracted from diesel particles by bovine serum albumin in solution, simulated lung surfactant, fetal calf serum (FCS), and physiologic saline. Only FCS was found to extract some mutagenic activity from the diesel particles. These investigators concluded that the mutagens in diesel particles would not be readily available *in vivo*. Similarly, Brooks et al. (1981) found only a small amount of mutagenic activity in serum or lavage fluid that was incubated with diesel exhaust particles. The conclusion that the mutagens in diesel particles would not be readily available *in vivo* (Siak et al., 1980) via particle extraction by the lung's extracellular lining fluid (ELF) lacks definitive experimental proof. The ELF is a complex mixture of constituents that undoubtedly have a broad range in hydrophobicity (George and Hook, 1984; Wright and Clements, 1987), and it fundamentally differs from serum in terms of chemical composition (Gurley et al., 1988). Moreover, assessments of the ability of lavage fluids, which actually

represent substantially diluted ELF, to extract mutagenic activity from diesel particles clearly do not reflect the *in vivo* condition. However, as will be discussed hereafter, some existing evidence does suggest that extraction of organic constituents from diesel particles by ELF prior to particle phagocytosis by lung phagocytes may be of relatively minor importance in mobilizing carcinogenic agents from the particles *in vivo*.

Another, more likely mechanism by which organic carcinogens, (e.g., polycyclic aromatic hydrocarbons) may be extracted from diesel particles in the lung is by particle dissolution within the phagosomes of alveolar macrophages (AM). Dissolution in macrophages presupposes that the particles are internalized by these phagocytes. Normally, the lung's AM can phagocytize particles, including diesel particles, that deposit in the peripheral air spaces within hours (Lehnert and Morrow, 1985; White and Garg, 1981). Some *in vitro* evidence (Strom, 1984) suggests that AM in the lungs of rats chronically exposed to diesel exhaust may not be markedly impaired in terms of phagocytic activity. On the other hand, Castranova et al. (1985) contend that chronic exposure to diesel exhaust depresses AM phagocytic activity; this latter interpretation, however, was based on the results of a chemiluminescence assay and not on direct quantitation of phagocytosis *per se*. Regardless, histologic observations of the lungs of animals subchronically or chronically exposed to diesel particles indicate that most of the retained particles are sequestered in particle-laden macrophages (Wiester et al., 1980; Plopper et al., 1983). Thus, a mechanism by which diesel particles can gain entry into the AM, and subsequently undergo dissolution therein, is available, at least under some exposure conditions. However, as high lung burdens of diesel particles are attained, such as during chronic, high atmospheric mass concentration exposures to diesel particles, AM that become heavily laden may reach their phagocytic capacity, and in this case, the abilities of the AM to further phagocytize deposited particles may be reduced. Additionally, the decreased mobility of AM that are filled with particles may diminish their migratory activities (Morrow, 1988; Lehnert et al., 1989) on the alveolar surface and their migration from one alveolus to another (Ferin, 1982). Furthermore, AM that reside as aggregates in the alveoli following chronic diesel exposure (White and Garg, 1981) would appear to have essentially no migratory activity. Decreases in particle-macrophage interactions would be an expected outcome of reduced levels of AM

migratory activity. Under these conditions, an increasing fraction of deposited particles could escape the phagocytic mechanism and thereby be relatively more abundantly available over time in the ELF prior to: (1) their removal from the lung by extra-macrophagic clearance via the tracheobronchial route, (2) their subsequent engulfment by newly recruited phagocytes. and/or 3) their engulfment by alveolar epithelial cells (i.e., Type I pneumocytes).

Specific details about the physicochemical conditions of the intraphagolysosomal environment where particle dissolution in AM presumably occurs *in vivo* have not been well characterized. However, it is known that the phagolysosomes constitute an acidic (pH 4 to 5) compartment in macrophages (Nilsen et al., 1988; Ohkuma and Poole, 1978). The relatively low pH in the phagolysosomes has been associated with the dissolution of some types of inorganic particles (e.g., some metals) by macrophages (Marafante et al., 1987; Lundborg et al., 1984), but quantitative information as to how organic constituents of diesel particles (e.g., B[a]P) may be extracted in the phagolysosomes is limited (Bond et al., 1983). Possibly, the containment of diesel particles in macrophages may in fact limit the solubilization of organic components while promoting their retention in the lung, with the end result being a prolonged exposure of the respiratory epithelium to the gradual release of carcinogenic agents. For example, Creasia and co-workers (1976) found that when crystalline B[a]P was instilled into the lungs of mice, it was removed from the respiratory tract with a half time ($t_{1/2}$) of ~1.5 h, but when the B[a]P was adsorbed to 0.5 to 1.0 μm carbon particles (a particle size range that would be expected to be readily phagocytized by resident AM shortly after deposition), its $t_{1/2}$ in the respiratory tract increased to ~36 h. Hence, the adsorption of BaP to the carbon particles increased the lung retention of the B[a]P greater than 20-fold. Similar results have also been obtained with BaP adsorbed onto other particle types, including insoluble Ga_2O_3 (Sun et al., 1982), insoluble ferric oxide (Saffiotti et al., 1964), and diesel particles (Sun et al., 1984). Consistent with a gradual elution of the B[a]P in AM, Creasia and co-workers (1976) also found that the removal of B[a]P when bound to the carbon was faster than the lung clearance of carbon particles only, which had a clearance $t_{1/2}$ of about 7 d. When the B[a]P was adsorbed onto carbon particles larger than would be expected to be easily phagocytized by AM (15 to 30 μm), the rates of elimination of the B[a]P and the larger particles from the lung were virtually identical. This

latter finding suggests that the elution of organics like B[a]P from particles by ELF may play a less important role in the extraction process than do the lung phagocytes.

Several investigators have shown that crystalline B[a]P has a relatively low carcinogenicity (Henry and Kaufman, 1973; Kuschner, 1968; Henry et al., 1973; Saffiotti et al., 1964), which may be due to its rapid elimination when unbound to particles. Henry and Kaufman (1974) also reported that the lung retention of B[a]P is prolonged as the carrier particle size is increased, and such increases in particle-associated retention of B[a]P have been correlated with enhanced carcinogenicity (Farrell and Davis, 1974). Such findings have been interpreted to suggest that the retardation in the lung clearance of an organic carcinogen is one of the major mechanisms by which particles can enhance their tumorgenicity (Creasia et al., 1976). Yet, Farrell and Davis (1974) found that a BaP-carbon particle combination in the particle size range of 0.5-1.0 mm was more tumorigenic than when the B[a]P was adsorbed to 15 to 30 μm size carbon particles; again, particles in this latter larger size range would not be readily endocytosed by resident AM, and, accordingly, would essentially be constantly in the presence of ELF. This observation indicates that the pulmonary retention of B[a]P *per se* is not a firm predictor of tumorigenicity. Rather, the above lines of information suggest that relatively continual availability of B[a]P via an extraction mechanism (i.e., extraction in the phagosomes of lung free cells) may be a primary determinant of carcinogenicity. As argued by Saffiotti et al. (1972), a carcinogen can be much more effective in producing a tumorigenic response when given in small divided doses over a period of time than when it is delivered as a single equivalent dose.

Quantitative predictions as to how readily carcinogenic organics may be extracted from diesel particles in human lung cannot be reliably extrapolated from existing animal data. As shown by Creasia et al (1976), the polycyclic aromatic hydrocarbon B[a]P adsorbed onto carbon particles is removed from the mouse lung at a rate that is ~5 times faster than the rate of clearance of the carbon particles only. For the rat, Sun and co-investigators (1984) have reported that the $t_{1/2}$ for the lung clearance of B[a]P adsorbed onto diesel particles over a period of time consistent with alveolar phase clearance was ~18 d. This latter value is similar to the $t_{1/2}$ for the removal of innocuous particles from the rat's lung during the early, more rapid component of alveolar phase clearance (Snipes et al., 1988;

Snipes et al., 1981; Ferin, 1982; Lehnert, 1988; Lehnert et al., 1989; Lehnert, 1989). These findings collectively suggest that the extraction of organic components from carrier particles by AM may differ among species, although differences in the lung burdens administered in the above investigations may have influenced their outcomes.

Comparative studies of human AM and AM from other species used in previous diesel exposure studies as to their relative abilities to extract carcinogens from diesel particles may provide useful information for human dosimetry estimates. Such investigations would ideally examine how the extraction process relates to the level of diesel particulate load in the AM. A subsequent dosimetry model would take into account, then, the kinetics of carcinogen mobilization by AM as a function of particulate burden, the kinetics of the metabolic fates of procarcinogens associated with diesel particles a la Bond et al (1984), and existing information that relates the amount of a material like B[a]P in the respiratory tract to its rate of removal (Medinsky and Kampcik, 1985). Additionally, studies directed to the relative abilities of human polymorphonuclear leukocytes (PMN) to extract organic constituents from diesel particles are also warranted in that diesel-containing PMN can become a major component of the lung's free cell population following chronic exposure to particulate diesel exhaust (Strom, 1984; White and Garg, 1981). In this same regard, further information as to how other lung cell types that can engulf deposited diesel particles (i.e., Type I pneumocytes, interstitial macrophages, and newly recruited blood monocytes) may kinetically extract procarcinogenic agents from the particles is required in order to better establish existing cellular bases for an extraction process. Such data could then be used in conjunction with cell type/estimated lung cell number data obtained from morphometric analyses of lungs exposed to diesel particles as an approach to quantitatively model potential cellular sites of extractions of organics from diesel particles. This approach, however, is further complicated by the likelihood that some fraction of the particulate burdens in lung cells may include particles that were previously contained in still other cells; hence, extractable organics in this fraction presumably would be less than that for particles that had no history of prior cell containment.

It should also be pointed out that studies designed to examine the extraction of organics by AM have generally focused on B[a]P as a representative procarcinogen

associated with diesel particles. Numerous other agents with carcinogenic activity are also associated with diesel particles; these chemical constituents may be extracted from particulate diesel exhaust with *in vivo* kinetics that differ more or less from those of B[a]P. Thus, existing dosimetry models that incorporate the dissolution of B[a]P from diesel particles as a representative organic constituent (Yu and Yoon, 1988) may not suitably reflect the actual bioavailability of other procarcinogenic agents on diesel particles that may contribute to the development of lung neoplasms.

8.5.3. Bioavailability of Adsorbed Compounds as a Function of Particle Clearance Rates and Extraction Rates of Adsorbed Compounds

The bioavailability of the organic compounds adsorbed to diesel soot particles is a function of desorption from the particles which is dependent, at least in part, upon the retention time of these particles in the alveolar region. The precise quantitative relationships between bioavailability and rates of clearance and desorption are unclear at present.

Sun et al. (1984) exposed F344 rats to 30 min, nose-only inhalation of diesel exhaust particles to which ^{3}H-B[a]P had been adsorbed (0.1 percent by mass). The rate limiting step for bioavailability of B[a]P was suggested to be the rate at which it was desorbed from the particle, and that the composition of the carrier particle and the associated physico-chemical interactions between the particle and the adsorbed organic were involved in this process. This postulation was justified by the fact that lung retention times for diesel exhaust particles and insoluble gallium oxide particles is quite long (62±14 and 65±16 d, respectively), yet lung retention of ^{3}H-B[a]P was much greater when associated with diesel exhaust particles.

Also of importance in assessing the rate of biovailability would be the rate and extent of phagolysosomal solubilization of adsorbed compounds. In analyzing phagolysosomal dissolution of various ions from particles deposited in the lungs of Syrian golden hamsters, Godleski et al. (1988) demonstrated that solubilization did not necessarily result in clearance of the ions and that binding of the solubilized components to cellular and extracellular structures occurred. It is reasonable to assume that phagocytized diesel soot particles may be subject to similar processes, and that these processes would be important in determining the rate of bioavailability of the particle-bound constituents of diesel exhaust. Perhaps

because of longer residence times for particle-adsorbed PAHs, the macrophages are better able to carry out some of the metabolic activation steps.

Pepelko (1987) in reviewing species variability in alveolar clearance of particulate matter provided an expression for the relationship among overall clearance rate ($t_{1/2b}$), the dissolution half-time ($t_{1/2s}$), and the particle removal half-time ($t_{1/2p}$):

$$t_{1/2b} = \frac{t_{1/2s} \times t_{1/2p}}{t_{1/2s} + t_{1/2p}} .$$

That lung clearance rate is the result of the combined effects of dissolution and AM-mediated transport from the lungs is indicated by this expression. Hence, for insoluble particles it follows that the half-time for AM-mediated transport would be similar to the actual lung clearance half-time. If particle clearance half-time ($t_{1/2p}$) is not experimentally derived, it can be estimated by rearrangement of the equation:

$$t_{1/2p} = \frac{t_{1/2b} \times t_{1/2s}}{t_{1/2s} - t_{1/2b}} .$$

If the dissolution half-time ($t_{1/2s}$) and the particle removal half-time ($t_{1/2p}$) are sufficient for accounting for clearance, then pulmonary retention can be calculated by first-order kinetics:

$$v = dR/dt = kR ,$$

where v is velocity, R is particulate matter concentration in the lungs, and k is the elimination rate constant (equal to ln $2/t_{1/2}$). Integration of this equation yields:

$$R = R_0 e^{-kt} ,$$

where R_0 is the initial particulate matter content of the lungs and t is time. Since dissolution of particulate matter and mechanical transport are concurrent, the retention can be expressed as:

$$R = 100e^{-(k_s + k_p)t} .$$

This expression does not account for delayed absorption following dissolution, transport to lymph nodes, reactions with macromolecules of the respiratory tract lining, or clearance by more than an exponential rate. However, if these factors are adjusted for or are not of significance, then the percentage of the remaining dose solubilized and absorbed each day can be obtained:

$$A = 100k_s e^{-(k_s+k_p)t} ,$$

where A is the daily absorption at time t. The total bioavailable dose can then be approximated by

$$\% \ absorption = \frac{100k_s}{k_p+k_s} 1-e^{-(k_m+k_s)t} .$$

8.5.4. Summary: Bioavailability of Particle-Adsorbed Agents.

The available studies provide animal data attesting to the importance of exhaust particles for increasing absorption potential of PAHs and the subsequent increase in bioavailability of these compounds over time due to retention of particles and particle overload conditions. The total exposure of the biological system to particle-associated organics will be a function of exposure duration, deposition and pulmonary clearance of the particles, the desorption of these organics from the particles, and the subsequent absorption of the organic compounds into the blood and tissues. Sun et al. (1984) suggested this process to be dependent upon the physicochemical interactions between the particle and its associated organics. Furthermore, the profile of absorbed organic constituents may be affected by alveolar macrophage-mediated biotransformation of these particle-associated organic compounds. Alveolar macrophage activity may also alter the potential bioavailable dose by relocation of ingested particles and their adsorbed organics to other pulmonary regions or lymphatic tissue. It is also important to note that macrophage-mediated metabolism of particle-associated organics is no doubt dependent upon the accumulation of

the particles by the macrophage. This may, in part, explain the greater effects noted for particle-associated vs. free PAHs. When a particle overload situation exists (as demonstrated by the studies summarized in Chapter 7), the time frame in which these processes can occur would also be increased. There do not appear to be any studies that provide definitive quantitative relationships among these processes.

8.6. INHIBITION OF RESPIRATION BY HIGH CONCENTRATIONS OF NOXIOUS AGENTS

Exposure to whole diesel exhaust will result in inhalation of components such as formaldehyde, acrolein, and sulfur dioxide, all of which have been demonstrated to be sensory irritants. The fact that these irritants also affect respiratory rates (Kane and Alarie, 1978, 1979) is of concern when considering inhalation exposure to diesel emissions. The sensory irritant-induced reduction of respiratory rate is mediated through stimulation of free nerve endings of the afferent trigeminal nerve (Ulrich et al., 1972). This physiologic reflex response has been shown to be a dose-dependent response (Alarie, 1966, 1973; Kane and Alarie, 1978).

A rapid (within 1 to 2 min), concentration-dependent decrease in respiratory rate followed by a rapid (within 12 min), concentration-dependent accommodation was reported by Alarie et al. (1973) for mice exposed to sulfur dioxide at concentrations of 17, 62, 123, or 298 ppm. This return to normal respiratory rates suggested that the sensory irritation response resulting in respiratory rate depression was undergoing a desensitization despite continued exposure. It was hypothesized that the SO_2 dissociated into SO_3^{2-} and HSO_3^- anions, both of which stimulate the free nerve endings of the trigeminal nerve. This concentration range encompasses the 20 to 40 ppm SO_2 content reported for diesel exhaust (see Chapter 2).

Kane and Alarie (1978) reported similar findings for acrolein-exposed mice, although the response to the irritant was slower. This varied response pattern initiated further experiments that examined the effects of mixtures of acrolein and sulfur dioxide (Kane and Alarie, 1979) and acrolein and formaldehyde (Kane and Alarie, 1978). In the 1978 study,

evaluation of the response patterns (rapidity, magnitude and duration of response, and accommodation period) indicated that each compound produced a characteristic response. Application of a mathematical model indicated that these interactions exhibited a competitive agonism between acrolein and sulfur dioxide. In the 1979 study, exposures to sulfur dioxide alone or to acrolein alone produced a maximum, but transient, reduction in respiratory rate of 36.1 percent and 51.6 percent, respectively. Each of these compounds produced a characteristic response pattern. However, exposure to mixtures of sulfur dioxide and acrolein produced response patterns indicating that either irritant could alter or completely negate the effect of the other. Generally, the recovery pattern following exposure to the mixtures was slower than that for either irritant alone.

Alarie (1973) using data of Murphy et al. (1963) and Amdur and Mead (1955) noted that nitro-olefins (possible reaction products of nitrogen oxides and olefinic hydrocarbons of exhaust gases) are more potent (estimated at 40 to 100-fold) sensory irritants than is sulfur dioxide, and that these considerations may be relevant in assessing effects of these pollutants on sensory irritation and alteration of respiratory rate.

8.7. CONSIDERATIONS FOR DOSIMETRY MODELING

It is evident from the material reviewed in this and preceding chapters that derivation of a dosimetry model for assessing the carcinogenic potential of diesel exhaust must consider many factors. A primary point of concern in this respect is the difficulty inherent in demonstrating evidence of carcinogenicity of a weak carcinogen, especially one that is delivered at very low exposure concentrations. Epidemiologic studies provide only limited and often controversial evidence. Experimental animal studies, however, have demonstrated a relationship between diesel exhaust exposure and increased tumor incidence, but employ higher exposure concentrations than would be normally encountered by humans. Furthermore, the uncertainty of animal-to-human extrapolation and interspecific variability in physiologic (respiratory rates, pulmonary capacities and volumes, respiratory tract anatomy, etc.) and response parameters (pulmonary clearance mechanisms and particle overloading, tumor incidence) must be considered in dosimetry modeling.

It is also necessary to consider if the observed increases in tumor incidences reported in animal studies are attributable to the organic constituents only, or if the particles to which they are adsorbed are also functionally involved and not acting merely as a vehicle. The function of the particulate matter may be twofold: (1) increasing lung epithelial cell proliferation rates or (2) increasing the retention of organic compounds. This latter function may be instrumental in explaining the increased tumor incidence in rats exposed to particle adsorbed organics (B[a]P) (Saffiotti et al., 1964, 1965; Henry and Kaufman, 1973; Henry et al., 1975) where a slow release of compound dose is delivered to the cells as opposed to administration of pure B[a]P which may overwhelm the cells' metabolizing capacities thus limiting the production of the reactive B[a]P metabolite. These factors would translate into a situation of competing risks in the dosimetry model (dose delivery rate vs. chronicity of exposure).

8.8. SUMMARY

Recent studies have provided evidence for tumorigenicity and carcinogenicity in laboratory animals following chronic inhalation exposure to whole diesel exhaust at particle concentrations as low as 3.5 mg/m^3. The pulmonary clearance of diesel exhaust particles is multiphasic and involves several processes including a relatively rapid mucociliary transport and slower macrophage-mediated processes. The observed dose-dependent increase in the particle burden of the lungs is due, in part, to an overloading of alveolar macrophage function. The resulting increase in particle retention has been shown to increase the bioavailability of particle adsorbed mutagenic and carcinogenic components such as B[a]P and 1-NP. Experimental data also indicate alveolar macrophage-mediated metabolism and phagolysosomal solubilization of particle-adsorbed components. Although macromolecular binding of diesel exhaust particle-derived PAH and the formation of DNA adducts following exposure to diesel exhaust have been reported, a quantitative relationship between these and increased carcinogenicity is not available.

In addition to the aforementioned points, one must also consider the fact that other compounds (e.g., gas-phase chemical irritants) may alter respiratory rate and, therefore, the

actual dose of potentially toxic components. Moreover, a better knowledge of particle dissolution rate and particle removal rate is necessary for more accurately assessing bioavailability of potentially carcinogenic components of diesel exhaust.

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9. EPIDEMIOLOGY STUDIES

9.1. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EMISSIONS

Emissions of diesel exhaust are made up of toxicants that include oxides of nitrogen and sulfur, carbon monoxide, and particulate matter consisting of a carbon core with many organic compounds, especially the polycyclic aromatic hydrocarbons absorbed on the surface. These emissions contain about 100 times more particulate matter than gasoline engine exhaust; the implications of this for human health are still unknown. To evaluate the risk assessment of the diesel exhaust emissions mortality and morbidity, studies on health effects of diesel emission exposures are reviewed here.

Three types of studies on the health effects of diesel emission exposures are considered: (1) cohort studies, (2) case-control studies of lung cancer, and (3) case-control studies of bladder cancer. Although an attempt was made to cover all the relevant studies, there are certain studies that are not included in this report for various reasons. Since the change from steam to diesel engines in locomotives was about 95 percent complete by 1959 and since diesel buses were introduced about the same time, studies conducted prior to 1959, when diesel exhaust exposure was less common, are not considered here (Raffle, 1957; Kaplan, 1959). As more definitive results were available in railroad workers, the pilot study in those workers (Schenker et al., 1984) is not reviewed here. Studies by Waxweiler et al. (1973), Gustafsson et al. (1986), Stern et al. (1981), and Garland et al. (1988) were excluded either because of uncertainty of exposure to diesel exhaust or because exposure was to both diesel and gasoline exhaust. For the first type of studies the cohorts of heavy construction equipment operators, railroad and locomotive workers, and bus garage employees were retrospectively studied to determine increased mortality and morbidity resulting from exposures to varying levels of diesel emissions at the work place.

The hypothesis-generating study by Buiatti et al. (1985) was excluded because it would have needed confirmation by a definitive study. The study by Coggon et al. (1984) (both case-control study of lung cancer as well as of bladder cancer) also was not included, as the

occupational information was abstracted from death certificates, that had not been validated; this would have resulted in limited information. A total of seven lung case control studies are considered in this report.

Case-control studies of bladder cancer by Vineis and Magnani (1985), Silverman et al. (1986), Jensen et al. (1987), and Risch et al. (1988) were not considered in this report either because the exposure was categorized as motor exhaust or because the occupations such as taxi, bus, and truck drivers were considered together as one job category and it was difficult to determine whether the observed effect was because of exposure to diesel or gasoline exhaust.

A total of five bladder cancer case-control studies are described in this report.

9.2. COHORT STUDIES

9.2.1. Waller (1981): Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes

A nonconcurrent prospective mortality study of a cohort of London transport workers was conducted to determine if there was an excess of deaths from lung cancer that could be attributed to diesel exhaust exposure. A total of nearly 20,000 male employees aged 45 to 64 were followed for a 25-yr period between 1950 and 1974 constituting a total of 420,700 man-years at risk. These were distributed among five categories: drivers, garage engineers, conductors, motormen or guards, and engineers (works). Most of them lived in the greater London area. Lung cancer cases occurring in this cohort were ascertained only from death certificates of individuals who died while still employed, or if retired, following diagnosis. Expected death rates were calculated by applying greater London death rates to the population at risk within each job category. Data were calculated in 5-yr periods and 5-yr age ranges, finally combining the results to obtain the total expected deaths in the required age range of 45 to 64 for the calendar period of 1950 to 1974. There were 667 cases of lung cancer reported compared with 849 expected to give a mortality ratio of 79 percent. In each of the five job categories, the observed numbers were below those expected. Engineers in garages had the highest mortality ratio (90 percent), but this did not differ significantly from

the other job categories. Environmental sampling was done at one garage, on 1 d, for benzo(a)pyrene concentrations and was compared with corresponding values recorded in 1957. Concentrations of benzo(a)pyrene recorded in 1957 were at least 10 times greater than those measured in 1979.

This study has several methodologic limitations. The lung cancer deaths ascertained for the study were the ones that occurred while in service (worker either died of lung cancer or retired after lung cancer was diagnosed). Although man-years at risk were based on the entire cohort, no attempt was made to trace or evaluate the individuals who had resigned from the London transport company for any other reason. Hence the information on resignees and lung cancer deaths among them was not available for analysis. This fact may have lead to a dilution effect, resulting in under- ascertainment of observed lung cancer deaths and underestimation of mortality ratios. Eligibility criteria for inclusion in the cohort, such as starting date and length of service with company were not specified. Because an external comparison group was used to obtain expected number of deaths, the resulting mortality ratios were less than 1; this may be a reflection of the "healthy worker effect". Investigators also did not categorize the five job categories by levels of diesel exhaust exposure nor did they use an internal comparison group to derive risk estimates.

The age range considered for this study was limited to 45 to 64 yr for the period between 1950 and 1964. It is not clear whether this age range was applied to calendar year 1950 or 1964 or at the mid-point of this 25-yr follow-up period. The rates were calculated by 5-year grouping but were not presented by 5-yr age group, which would have been more meaningful. No analyses were presented either by latency or by duration of employment (surrogate for exposure). The environmental survey based on benzo(a)pyrene concentrations suggests that the cohort in their earlier years were exposed to much higher concentrations of environmental contaminants than current concentrations. It would have been useful to examine lung cancer deaths at different time periods. Lastly, no data were collected on smoking habits.

9.2.2. Howe et al. (1983): Cancer Mortality (1965-1977) in Relation to Diesel Fume and Coal Exposure in a Cohort of Retired Railroad Workers

This is a retrospective cohort study of the mortality experience of 43,826 male pensioners of the Canadian National Railroad (CNR) between 1965 and 1977. Members of this cohort consisted of male CNR pensioners who had retired before 1965 and who were known to be alive at the start of that year, as well as those who retired between 1965 and 1977. The records were obtained from a computer file, which is regularly updated, that is used by the company for payment of pensions. To receive a pension each pensioner must provide, on a yearly basis, evidence to the effect that he is alive. Specific cause of death among members of this cohort was ascertained by linking these records to the Canadian Mortality Data Base which contains records of all deaths registered in Canada since 1950. Of the 17,838 deaths among members of the cohort between 1965 and 1977, 16,812 (94.4 percent) were successfully linked to a record on the mortality file. A random sample manual check on unlinked data revealed that failure to link was mainly due to some missing information on the death records.

Occupation at time of retirement was used by the Department of Industrial Relations to classify workers into three diesel fume and coal dust exposure categories. Nonexposed, possibly exposed, and probably exposed. Person-years of observation were calculated and classified by age at observation in 5-yr age groups (35-39, 40-44 --- 80-84, ≥85). The observed deaths were classified by age at death for different cancers, for all cancers combined, and for all causes of death combined. Standard mortality ratios (SMR) were then calculated using rates of the Canadian population for the period between 1965 to 1977.

Both total mortality (SMR = 0.95, $p < 0.001$) and all cancer deaths (SMR = 0.99, $p > 0.05$) were close to that expected for the entire cohort. Analysis by exposure to diesel fume levels in the three categories (nonexposed, possibly exposed, and probably exposed) revealed an increased relative risk for lung cancer among workers with increasing exposure to diesel fumes. The relative risk for nonexposed workers was presumed to be 1.0; for those possibly exposed the relative risk was elevated to 1.2, which was statistically significant

(p = 0.013); for those probably exposed it was elevated to 1.35 which was statistically highly significant (p = 0.001). The corresponding rates for exposure to varying levels of coal dust were very similar at 1.00, 1.21 (p = 0.012), and 1.35 (p = 0.001), respectively. The trend tests were highly significant for both exposures (p <0.001). Since there was considerable overlap between occupations involving probable exposure to diesel fumes and probable exposure to coal dust and since most members of the cohort were employed during the years in which the transition from coal to diesel occurred, it was difficult to distinguish whether lung cancer was associated with exposure to coal dust or diesel fumes.

Although this study showed a highly significant dose-response relationship with diesel fumes and lung cancer, it has several methodological limitations. There were concurrent exposures to both diesel fumes and coal dust during the transition period, therefore, misclassification of exposure may have occurred, since only occupation at retirement was available for analysis. It is possible that the elevated response observed for lung cancer was due to the combined effect of exposure to both coal dust and diesel fumes and not just one or the other. No information was provided on duration of employment in either diesel work or the coal dust-related jobs for other than those jobs held at retirement. Therefore, it was not possible to evaluate whether this omission would have led to an under- or overestimate of the true relative risk. Furthermore, a lack of information on potential confounders such as asbestos exposure and smoking makes the interpretation of the excess risk of lung cancer even more difficult. Information on cause of death was acquired from the mortality data linkage. There is a possibility that the cause of death may have been misclassified because of miscoding of the underlying cause of death. Further, it is not clear whether or not the investigators verified the cause of death which could also lead to misclassification. Again, it is difficult to evaluate whether this may have lead to under- or overestimation of the risk. Because of the above limitations, the findings from this study, at best, are only suggestive and not confirmatory of an excess risk of lung cancer from occupational exposure to diesel fumes.

9.2.3. Rushton et al. (1983): Epidemiological Survey of Maintenance Workers in the London Transport Executive Bus Garages and Chiswick Works

This is a nonconcurrent mortality cohort study of male maintenance workers employed for at least one continuous year between January 1, 1967 and December 31, 1975, at 71 London transport bus garages (also known as rolling stock) and at Chiswick Works. For all men the following information was obtained from computer listings: surname with initials, date of birth, date of joining company, last or present jobs, and location of work. For those individuals who left their job, date of and reason for leaving were also obtained. For those who died in service or after retirement and for men who had resigned, full name and last known address were obtained from an alphabetical card index in the personnel department. Additional tracing of individuals who had left was carried out through social security records. The area of their residence was assumed to be close to their work; therefore their place of work was coded as their residence. There were 100 different job titles that were coded into 20 broader groups. The reason for leaving was coded as died in service, retired, or other. The underlying cause of death was coded using the 8th revision of the International Classification of Dieases (ICD). Person-years were calculated from date of birth and dates of entry to and exit from the study using the man-years computer language program. These were then subdivided into 5-yr age and calendar period groups. The expected number of deaths were calculated by applying the 5-yr age and calendar period death rates of the comparison population to the person-years of corresponding groups. The mortality experience of the male population in England and Wales was used as the comparison population. Significance values were calculated for the difference between the observed and expected deaths, assuming a Poisson distribution.

There were a total of 50,008 person-years of observation in the study with a mean follow-up of 5.9 yr. Only 2.2 percent of the men were not traced. Observed deaths from all causes were significantly lower than expected (observed/expected = 495/607.46, $p < 0.001$). The observed deaths from all neoplasms and cancer of the lung were approximately the same as those expected. The only significant excess observed for cancer of the liver and gall bladder at Chiswick Works was based on four deaths ($p < 0.05$). A few

job groups showed a significant excess of risks for various cancers. Cancer of the liver and gall bladder (observed = 2, p <0.01) and cancer of the bladder (Observed = 3, p <0.01) were significant in the job categories of inspector and progress hand, respectively. Welders showed an excess of lung cancer (Observed = 3, p <0.05), bus mechanics had an excess of cancer of the brain (Observed = 4, p <0.04), and painters had an excess of bladder cancer (Observed = 2, p <0.02). All the excess deaths observed for the various job groups were based on very small numbers (usually smaller than 5) and merited cautious interpretation.

This is an exploratory mortality study of London transport maintenance workers that did not demonstrate any cancer excesses based on a large number of cases, which needs further exploration. Its several limitations include the small sample size, short duration of follow-up (average of only 6 yr), and lack of long enough latency period. Thus, the number of deaths by different causes and among the various job groups was too small to allow any meaningful conclusions. Details of work history were not obtained to permit any analysis by diesel exhaust emission exposure. Death information was ascertained from death certificates with inherent problems of inaccuracy, misdiagnosis, and errors in coding. It was not known whether a trained nosologist coded the death certificates or not. No adjustments were made for the confounding effects of smoking and socioeconomic factors.

9.2.4. Wong et al. (1985): Mortality Among Members of a Heavy Construction Operators Union With Potential Exposure to Diesel Exhaust Emissions

This is a historical prospective mortality study conducted on a cohort of 34,156 male members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. Study cohort members were identified from records maintained at Operating Engineers' Local Union No. 3-3A in San Francisco. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 yr of membership in this union between January 1, 1964, and December 31, 1978, were included in the study. Work histories of the cohort were obtained from job dispatch computer tapes. The study follow-up period was from January 1964 to December 1978. Death information was obtained from a trust fund, which provided information on retirement dates, vital status, and date of death for those who were entitled to retirement

and death benefits. The average duration of membership was 15 yr. As of December 31, 1978, there were 15,735 cohort members who were active members of the union, 10,505 (30.8 percent) who were inactive or terminated, 6670 (19.6 percent) retired, and 1238 (3.6 percent) who had died while active or retired. Vital status of 10,505 members who left the union was ascertained from the Social Security Administration. Death certificates were obtained from appropriate state health departments. Altogether, 3345 deaths in the cohort were coded using the ICD-7. Expected deaths and SMRs were calculated using the U.S. national age-sex-race cause-specific mortality rates for 5-yr time periods between 1964 and 1978. The entire cohort population contributed to 372,525.6 person-years in this 14-yr study period. Death certificates could not be obtained for 102 individuals; these individuals were included in the calculation of the SMR for all causes of death but were deleted from the cause-specific SMR analyses.

A total of 3345 deaths was observed compared with 4109 expected. The corresponding SMR for all causes was 81.4 (p = 0.01), which confirmed the "healthy worker effect". Eight hundred seventeen deaths were attributed to malignant neoplasms, slightly fewer than the expected 878.34 based on the U.S. white male cancer mortality rates (SMR = 93.0, p = 0.05). Mostly there were SMR deficits for cause-specific cancers, including lung cancer for the entire cohort (SMR = 98.6, observed = 309). The only significant excess of SMR was observed for cancer of the liver (SMR = 166.7, observed = 23, p <0.05). There were a couple of statistically nonsignificant cancer excesses observed for cancer of the bladder (SMR = 118.1, observed = 27) and cancer of the stomach (SMR = 117.6, observed = 44). For nonmalignant causes statistically significant (p <0.01) excesses were observed for emphysema (SMR = 165.3, observed = 116) and accidental deaths (SMR = 127.0, observed = 348).

Analysis by length of union membership as a surrogate of duration for potential exposure showed statistically significant (p <0.01) increases in SMRs of cancer of the liver (SMR = 424.1) in the 10 to 14-yr membership group, emphysema in the 15 to 19 and 20+ year membership groups, SMRs being 174.5 and 174.8, respectively, and accidental deaths in the 10 to 14-yr membership group (SMR = 127.7). In addition to these increases, a statistically significant (p <0.05) increase was also observed for cancer of the stomach

(SMR = 248.4) in the 5 to 9-yr membership group. For emphysema a positive trend was observed with increasing length of membership. Although the SMR for cancer of the lung had a statistically significant deficit in the less than 5-yr duration group, it showed a positive trend with increasing length of membership which, leveled off after 10 to 14 yr.

Cause-specific mortality analysis by latency period showed a positive trend for SMRs of all causes of death, although all of them were statistically significant deficits, reflecting the diminishing "healthy worker effect". This analysis also demonstrated statistically significant SMR excesses for cancer of the liver (10 to 19 yr group, SMR = 257.9), emphysema (20+ yr group, SMR = 199.4), and accidents (10 to 19 yr group, SMR = 140.6). A positive trend with increasing latency was observed for emphysema. The SMR for cancer of the lung showed a statistically significant deficit for the 10-yr follow-up, but showed a definite positive trend with increasing latency.

In addition to these analyses of the entire cohort, similar analyses were carried out in various subcohorts. Analyses of retirees, 6678 individuals contributing to 32,670.1 person-years, showed statistically significant increases ($p < 0.01$) in SMR for all cancers (SMR = 145.3, observed = 389), all causes of death (SMR = 114.5, observed = 1,345), cancer of the digestive system (SMR = 142.4, observed = 103), cancer of the large intestine (SMR = 181.8, observed = 46), cancer of the respiratory system (162.4, observed = 161), cancer of the lung (SMR = 164.1, observed = 155), emphysema (SMR = 277.3, observed = 75), and cirrhosis of the liver (SMR = 173.5, observed = 38). The other two significant excesses ($p < 0.01$) were for lymphosarcoma and reticulosarcoma (SMR = 231.2, observed = 10) and nonmalignant respiratory diseases (SMR = 129.0, observed = 112). Further analysis of the 4075 retirees (18,677.8 person-years) who retired at age 65 or who retired earlier but had reached the age of 65, revealed statistically significant SMR increases for all cancers (SMR = 114.7, observed = 224, $p = 0.05$), cancer of the lung (SMR = 130, observed = 86, $p < 0.05$), and lymphosarcoma and reticulosarcoma (SMR = 266.5, observed = 8, $p < 0.05$).

To analyze cause-specific mortality by job held (potential exposure to diesel exhaust emissions), 20 functional job titles were used, which were further grouped into three potential categories: (1) high exposure, (2) low exposure, and (3) unknown exposure. A person was classified in a job title if he ever worked on that job. Based on this classification

system, if a person had ever worked in a high-exposure job title he was included in that group, even though he may have worked for a longer time in a low-exposure group or in an unknown exposure group. Information on length of work in any particular job, hence indirect information on potential length of exposure, was not available either.

For the high-exposure group a statistically significant excess was observed for bulldozer operators who had 15 to 19 yr of membership and 20+ yr of follow-up for cancer of the lung (SMR = 343.4, $p < 0.05$). This excess was based on 5 out of 495 deaths observed in this group of 6712 individuals who contributed 80,327.6 person-years of observation.

The cause-specific mortality analysis in the lowdiesel exhaust exposure group revealed statistically significant SMR excesses in individuals who had ever worked as engineers. These excesses were for cancer of the large intestine (SMR = 807.2, observed = 3, $p < 0.05$) among those with 15 to 19 yr of membership and length of follow-up of at least 20 yr, and cancer of the liver (SMR = 871.9, observed = 3, $p < 0.05$) among those with 10 to 14 yr membership and length of follow-up of 10 to 19 yr. There were 7032 individuals who contributed to 78,402.9 person-years of observation in the low diesel exposure group.

For the unknown exposure group a statistically significant SMR was observed for motor vehicle accidents only (SMR = 173.3, observed = 21, $p < 0.05$). There were 3,656 individuals who contributed to 33,388.1 person-years of observation in this category.

No work histories were available for those who started their jobs before 1967 and for those who held the same job prior to and after 1967. This constituted 9,707 individuals (28 percent of the cohort) contributing to 104,447.5 person-years. Statistically significant SMR excesses were observed for all cancers (SMR = 112, observed = 339, $p < 0.05$) and cancer of the lung (SMR = 119.3, observed = 141, $p < 0.01$). A significant SMR elevation was also observed for cancer of the stomach (SMR = 199.1, observed = 30, $p < 0.01$). There were statistically significant SMR elevations at $p < 0.05$ for 3 non-carcinogenic causes, benign tumors, emphysema, and non-motor-vehicle accidents.

This study demonstrates a statistically significant excess for cancer of the liver, but also shows statistically significant deficits in cancers of the large intestine and rectum. It may be, as the authors suggested, that the liver cancer cases were actually cases resulting from metastases from the large intestine and/or rectum, since tumors of these sites will frequently

metastasize to the liver. The excess in liver cancer mortality and the deficits in mortality that are due to cancer of the large intestine and rectum could also, as the authors indicate, be due to misclassification. Both possibilities have been considered by the investigators in their discussion.

An important finding of the study is the positive trend by latency period as well as by length of membership for SMR of emphysema. Emphysema is usually associated with smoking, and with exposure to heavy dust, fumes, and gases. As most of these heavy construction equipment workers work outdoors at construction sites, there is a lot of occupational exposure to various kinds of heavy dusts as well as exhaust fumes, and thus it is not known what the contribution is, if any, of diesel exhaust to emphysema risk. The confounding by smoking cannot be ruled out either, although a smoking habits survey was carried out. This smoking survey has some limitations, however, which are described at the end of the study.

Cancer of the lung also showed a positive trend with length of membership as well as with latency although none of the SMRs were statistically significant except for the workers without any work histories. The individuals without any work histories may have been the ones who were in their jobs for the longest period of time, since workers without job histories included those who had the same job before and after 1967 and thus may have worked 12 to 14 yr or longer. If they had belonged to the category where heavy exposure to diesel exhaust emissions was very common for this prolonged time, then the increase in lung cancer as well as stomach cancer might be linked to diesel exhaust. Further information on those without work histories should be obtained if possible since such information may be quite informative with regard to the evaluation of the carcinogenicity of diesel exhaust.

The study design is adequate, covers a long observation period, has a large enough population, and is appropriately analyzed; however, it has too many limitations to permit any conclusions. First, there are no exposure histories available. One has to make do with the job histories which provide limited information on exposure level. Any person who ever worked at the job or any person working at the same job over any period of time are included in the same category; this would have a dilution effect, since extremely variable

exposures were considered in the study. The length of time worked in any particular job is not available. Work histories were not available for 9707 individuals who contributed 104,447.5 person-years, a large proportion of the study cohort (28 percent). These individuals happen to show the most evidence of a carcinogenic effect. Confounding by alcohol consumption for cancer of the liver and smoking for emphysema and cancer of the lung were not ruled out. Though 34,156 members were eligible for the study, the vital status of 1765 individuals was unknown. Nevertheless, they were still considered in the denominator of all the analyses. The investigators fail to mention how the person-year calculation for these individuals was handled. Also, some of the person-years might have been overestimated, as people may have paid the dues for a particular year and then left work. These two causes of overestimation of the denominator may have resulted in some or all the SMRs to be underestimated.

As for the smoking survey the investigators took a very small sample (133 out of 34,156, which was not even 1 percent). Of 133, only 107 (80 percent) participated. It was a systematic sample, but the authors have neglected to mention how the list was prepared for this systematic sample. Hence, the sample may not be representative of the study population, and, with a small sample size the results are not generalizable. Lastly, the questionnaire asked only for the current smoking history. No detailed history was obtained for the amount smoked or length of smoking history, both of which have bearing on emphysema as well as lung carcinoma.

9.2.5. Edling et al. (1987): Mortality Among Personnel Exposed to Diesel Exhaust

This is a cohort study of bus company employees, which investigated a possible increased mortality in cardiovascular diseases and cancers from diesel exhaust exposure. The cohort comprised all males employed in five different bus companies in southeastern Sweden between 1950 and 1959. The total cohort of 694 men (after loss of 5 men to follow-up) were divided into three exposure categories: clerks with the lowest exposure, bus drivers with moderate exposure, and bus garage workers with highest exposure. Using information from personnel registers, individuals were classified into one or more categories and could

have contributed person-years at risk in more than one exposure category. The study period was from 1951 to 1983; information was collected from the National Death Registry, and copies of death certificates were obtained from the National Bureau of Statistics. Workers who died after age 79 were excluded from the study because diagnostic procedures were likely to be more uncertain at higher ages (according to investigators). The cause-, sex-, and age-specific national death rates in Sweden were applied to the 5-yr age categories of person-years of observation to determine expected deaths for all causes, malignant diseases, and cardiovascular diseases. A Poisson distribution was used to calculate *p*-values and confidence limits for the ratio of observed to expected deaths.

The 694 men provided 20,304 person-years of observation with 195 deaths compared to 237 expected. A deficit in cancer deaths largely accounted for this lower- than-expected mortality in total cohort. Among subcohorts, no difference between observed and expected deaths for total mortality, total cancers, or cardiovascular causes was observed for clerks (lowest diesel exposure), bus drivers (moderate diesel exposure), and garage workers (high diesel exposure). The risk ratios for all three categories were less than 1 except for cardiovascular diseases among bus drivers which was 1.1.

When the analysis was restricted to members who had at least a 10-yr latency period and either any exposure or an exposure exceeding 10 yr, similar results were obtained with fewer neoplasms than expected while cardiovascular diseases showed risk around or slightly above unity.

The small size of the cohort and poor data on diesel exhaust exposure are among the major limitations of this study. Although lifetime occupational history was available, no industrial hygiene data were presented to validate the classification of workers into low, moderate, and high exposure to diesel exhaust based on job title. The power of the present study was estimated to be 80 percent to detect a relative risk of 1.2 for cardiovascular diseases and 1.4 for cancers, but for specific cancer sites, the power was much lower than this. No information was available on confounding effects of smoking and asbestos exposure at the work sites. In view of these serious limitations, there is insufficient evidence from the study to confirm or refute the hypothesis that exposure to diesel exhaust is associated with an increased risk of cancer and cardiovascular diseases.

9.2.6. Boffetta and Stellman (1988): Diesel Exhaust Exposure and Mortality Among Males in the American Cancer Society Prospective Study

Boffetta and Stellman conducted a mortality analysis of 46,981 males whose vital status was known at the end of the first 2-yr follow-up. The analysis was restricted to males aged 40 to 79 yr in 1982 who enrolled in the American Cancer Society's prospective mortality study of cancer. Mortality was analyzed in relation to exposure to diesel exhaust exposure and to employment in selected occupations related to diesel exhaust exposure. In 1982, more than 77,000 American Cancer Society volunteers enrolled over 1,200,000 men and women from all 50 states, the District of Columbia, and Puerto Rico in a long-term cohort study, the Cancer Prevention Study II (CPS-II). Enrollees were usually friends, neighbors, or relatives of the volunteers; enrollment was by family groups with at least one person in the household 45 yr of age or older. Subjects were asked to fill out a four-page confidential questionnaire and return it in a sealed envelope. The questionnaire included history of cancer and other diseases; use of medications and vitamins; menstrual and reproductive history; occupational history; and information on diet, drinking, smoking and other habits. There were three questions on occupation: (1) current occupation, (2) the last occupation, if retired, and (3) the job held for the longest period of time, if different from the other two. Occupations were coded to an ad hoc two-digit classification in 70 categories. Exposures at work or in daily life to any of the 12 groups of substances were also ascertained. These included diesel engine exhausts, asbestos, chemicals/acids/solvents, dyes, formaldehyde, coal or stone dusts, and gasoline exhausts. Volunteers checked whether their enrollees were alive or dead and recorded the date and place of all deaths every second year through. Death certificates were then obtained from state health departments and coded according to a system based in the ICD-9 by a trained nosologist.

The data were analyzed to determine the mortality for all causes and lung cancer in relation to diesel exhaust exposure, mortality for all causes and lung cancer and employment in selected occupations with high diesel exhaust exposure, and mortality from other causes in relation to diesel exhaust exposure. The incidence-density ratio was used as a measure of association, and test-based confidence limits were calculated by the Miettinen

method. For stratified analysis, the Mantel-Haenszel method was used for testing linear trends. Data on 476,648 subjects comprising 939,817 years of person risk were available for analysis. Three percent of the subjects (14,667) had not given any smoking history, and 20 percent (98,026) of them did not give information on diesel exhaust exposure and were therefore excluded from the main diesel exhaust analysis. Among individuals who had provided diesel exhaust exposure history, 62,800 were exposed and 307,143 were not exposed. Comparison of the population with known information on deisel exhaust exposure with the excluded population with no information on deisel exhaust exposure showed that the mean ages were 54.7 and 57.7 yr, the non-smokers were 72.4 percent and 73.2 percent, and the total mortality rates/1000/yr were 23.0 percent and 28.8 percent, respectively.

The all cause mortality was elevated among railroad workers [RR (relative risk) = 1.43, 95 percent CI = 1.2, 1.72], heavy equipment operators [RR = 1.7, 95 percent CI (confidence internal) = 1.19, 2.44], miners (RR = 1.34, 95 percent CI = 1.06, 1.68), and truck drivers (RR = 1.19, 95 percent CI = 1.07, 1.31). For lung cancer mortality the risks were significantly elevated for miners (RR = 2.67, 95 percent CI = 1.63, 4.37) and heavy equipment operators (RR = 2.60, 95 percent CI = 1.12, 6.06). Risks were also elevated but not significantly for railroad workers (RR = 1.59, 95 percent CI=0.94, 2.69) and truck drivers (RR = 1.24, 95 percent CI=0.93, 1.66). These risks were calculated according to the Mantel-Haenszel method, controlling for age and smoking. Although relative risk was nonsignificant for truck drivers, a small dose-response effect was observed when duration of diesel exhaust exposure for them was examined. For drivers who worked for 1 to 15 yr RR was 0.87, while for drivers who worked for more than 16 yr the RR was 1.33 (95 percent CI = 0.64, 2.75). Relative risks for lung cancer were not presented for other occupations. Mortality analysis for other causes and diesel exhaust exposure showed a significant excess of deaths (p <0.05) in the following categories: cerebrovascular disease, arteriosclerosis, pneumonia, influenza, cirrhosis of the liver, and accidents.

The two main methodologic concerns in this study are the representativeness of the study population and the quality of information on exposure. The sample, though very large, was comprised of volunteers thus the cohort, was healthier and less frequently exposed to important risk factors such as smoking and alcohol. Self-administered questionnaires were

used to obtain data on occupation and diesel exhaust exposure. None of this information was validated. Nearly 20 percent of the individuals had an unknown exposure status to diesel exhaust, and they experienced a higher mortality for all causes and lung cancer than both the diesel exhaust exposed and unexposed groups. This could have introduced a substantial bias in the estimate of the association. While only 0.8 percent of the subjects were lost to follow-up, the use of death certificates alone as a source of medical information poses problems in accuracy and coding. But the authors report that cancer deaths are routinely checked by histological confirmation from physicians or cancer registries. Given the fact that all the diesel exhaust exposure occupations such as heavy equipment operators, truck drivers, and railroad workers showed elevated lung cancer risk this study suggests a causal association between the two.

9.2.7. Garshick et al. (1988): A Retrospective Cohort Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers

An earlier case-control study of lung cancer and diesel exhaust exposure in U.S. railroad workers by these investigators had demonstrated a relative odds of 1.41 (95 percent CI = 1.06, 1.88) for lung cancer with 20 yr of work in diesel exhaust exposure jobs. To confirm these results a large retrospective cohort study was conducted by the same investigators. Data sources for the study were the work records of the U.S. Railroad Retirement Board (RRB). The cohort was selected based on job titles in 1959, which was the year by which 95 percent of the locomotives in the United States were diesel powered. Diesel exhaust exposure was considered to be a dichotomous variable depending on yearly job codes between 1959 and death or retirement through 1980. Industrial hygiene evaluations and descriptions of job activities were used to classify jobs as exposed or unexposed to diesel emissions. A questionnaire survey of 534 workers at one of the railroads was used to validate this classification. Employed workers 40 to 64 yr of age starting work between 1939 and 1949 in the sampled job codes in 1959 and eligible for railroad benefits were selected for the study. To qualify for benefits, a worker must have 10 yr or more of service with the railroad and should not have worked for more than 2 yr in a non-railroad job after leaving railroad work. Workers with recognized asbestos exposure

because of passenger car, steam pipe, and railroad building construction and repairs were not included among the job categories selected for study. However, a few jobs with some potential for asbestos exposure were included in the cohort, and the analysis was done with and without them.

The death certificates on all subjects identified in 1959 and reported by the RRB to have died through 1980 were searched. Twenty-five percent of them were obtained from the RRB and the remainder from the appropriate state department of health. Coding of cause of death was done without knowledge of exposure history, according to the ICD-8. If the underlying cause of death was not lung cancer, but was mentioned on the death certificate, it was assigned as a secondary cause of death so that the ascertainment of all cases was complete. Workers not reported by the RRB to have died by December 31, 1980, were considered to be alive. Deceased workers for whom death certificates had not been obtained, or if obtained did not indicate cause of death, were assumed to have died of unknown causes.

Proportional hazard models were fitted that provided estimates of relative risk for death caused by lung cancer using the partial likelihood method described by Cox, and 95 percent confidence intervals were constructed using the asymptotic normality of the estimated regression coefficients of the proportional hazards model. Exposure was analyzed by diesel exhaust-exposed jobs in 1959 and by cumulative number of years of diesel exhaust exposure through 1980. Directly standardized rate ratios for deaths from lung cancer were calculated for diesel exhaust exposed compared with unexposed for each 5-yr age group in 1959. The standardized rates were based on the overall 5-yr person-year time distribution of individuals in each age group starting in 1959. The only exception to this was between 1979 and 1980, when 2-yr person-year distribution was used. The Mantel-Haenszel analogue for person-year data was used to calculate 95 percent confidence intervals for the standardized rate ratios.

The cohort consisted of 55,407 workers; there were 19,396 deaths by the end of 1980. Death certificates were not available for 11.7 percent of all deaths. Of 17,120 deaths for whom death certificates were obtained, 48.4 percent were attributable to diseases of the circulatory system while 21 percent were attributable to all neoplasms. Of all neoplasms 8.7

percent (1694 deaths) were due to lung cancer. A higher proportion of workers in the younger age groups, mainly brakemen and conductors, were exposed to diesel exhaust while a higher percentage of workers in the older age groups were potentially exposed to asbestos.

In a proportional hazards model, analyses by age in 1959 found a relative risk of 1.45 (95 percent CI = 1.11, 1.89) among the age group 40 to 44 yr and a relative risk of 1.33 (95 percent CI = 1.03, 1.73) for the age group 45 to 49 yr. Risk estimates in the older age groups 50 to 54, 55 to 59, and 60 to 64 yr were 1.2, 1.18, and 0.99, respectively, and were not statistically significant. The two youngest age groups in 1959 had workers with the highest prevalence and longest duration of diesel exhaust exposure and lowest exposure to asbestos. When potential asbestos exposure was considered as a confounding variable in a proportional hazards model, the estimates of relative risk for asbestos exposure were all near null value and not significant. Analysis of workers exposed to diesel exhaust in 1959 (n = 42,535) excluding the workers with potential past exposure to asbestos, yielded the relative risks of 1.57 (95 percent CI = 1.19, 2.06) and 1.34 (95 percent CI = 1.02, 1.76) in 40 to 44 yr and 45 to 49 yr age groups in 1959. Directly standardized rate ratios were also calculated for each 1959 age group based on diesel exposure in 1959. The results obtained confirmed those obtained by using the proportional hazards model.

Relative risk estimates were then obtained using duration of diesel exhaust exposure as a surrogate for dose. In a model that used years of exposure up to and including exposure in the year of death, no exposure duration-response relationship was obtained. When analysis was done by disregarding exposure in the year of death and 4 yr prior to death, the risk of dying from lung cancer increased with the number of years worked in a diesel exposed job. In this analysis, diesel exposure was analyzed by exposure duration groups and in a model entering age in 1959 as a continuous variable. The workers with greater than 15 yr of exposure had a relative risk of lung cancer of 1.72 (95 percent CI = 1.27, 2.33). The risks for 1 to 4 yr of cumulative exposure was 1.20 (95 percent CI = 1.01, 1.44), for 5 to 9 yr of cumulative exposure it was 1.24 (95 percent CI = 1.06, 1.44), and for 10 to 14 yr of cumulative exposure it was 1.32 (95 percent CI = 1.13, 1.56). Directly standardized rate ratios were also calculated for each 1959 age group based on diesel

exposure in 1959. The results obtained were confirmatory to those obtained by using the proportional hazards model.

The results of this study demonstrating a positive association between diesel exhaust exposure and increased lung cancer are consistent with the results of the case-control study conducted by the same investigators in railroad workers dying of lung cancer from March 1981 through February 1982. This cohort study has addressed many of the weaknesses of the other epidemiologic studies. The large sample size (60,000) allowed sufficient power to detect small risks and also permitted the exclusion of workers with potential past exposure to asbestos. The stability of job career paths in the cohort ensured that of the workers 40 to 44 yr of age in 1959 classified as diesel exhaust exposed, 94 percent of the cases were still in diesel exhaust exposed jobs 20 yr later.

One of the limitations of this study was the lack of qualitative and quantitative data on exposure. Number of years exposed to diesel exhaust was used as a surrogate for dose. Another limitation of this study was the inability to examine the effect of years of exposure and latency. The use of death certificates and the changes in diagnosis of lung cancer from 1959 through 1980 was a methodologic consideration left unaddressed by the authors. Despite these limitations, the results of this study support the hypothesis that occupational exposure to diesel exhaust is associated with a modest risk (1.5) of lung cancer.

9.3. CASE CONTROL STUDIES OF LUNG CANCER

9.3.1. Williams et al. (1977): Associations of Cancer Site With Occupation and Industry From the Third National Cancer Survey Interview

This paper reports findings of the analysis of the Third National Cancer Survey (TNCS). The lifetime histories, occupations, and industries were studied for associations with specific cancer sites and types after controlling for age, sex, race, education, use of cigarettes or alcohol, and geographic location. A total of 7,518 incident invasive cancers occurring in the three years surveyed by the TNCS were interviewed. These comprised 57 percent of those eligible to participate. The interview included items on use of tobacco and alcohol (by type, amount, and duration), family income, patient education, and employment

history. Actual descriptions of the occupation and industry were recorded by interviewers, and according to the 1970 Census Coding Scheme were coded separately for main lifetime employment, recent employment, and other jobs held. Occupations or industries were combined to form larger groups. Coding of occupational and industrial labels in meaningful job categories was done by one of the authors. Of the 3539 interviewed males and 3937 interviewed females 95 percent and 84 percent listed some main employment, respectively. The basic analysis consisted of an intercancer comparison and involved comparing the proportions of specific main lifetime industries and occupations among patients with cancer at one site with those of patients having cancer at other sites combined as a control group, and this was done using a series of Mantel-Haenszel stratified contingency table analyses to yield odds ratios and Chi-Square values. Odds ratios (OR) were computed controlling for age, race, education, tobacco, alcohol, and geographic location, and these were done separately for males and females.

An excess risk for males for lung cancer was observed for the following main industrial groups; mines [OR = 1.21], construction (OR = 1.24), transportation (OR = 1.17), utility and sanitary services (OR = 2.79, $p < 0.05$), and professional (OR = 1.41). An excess of bladder cancer was reported for the mining industry (OR = 1.61). For females an excess of lung cancer was detected for the transportation industry (OR = 1.96); finance and retail industry (OR = 1.73); and the business, car repair, and miscellaneous service industry (OR = 2.29). None of these excesses were statistically significant. The transportation industry for males and females also showed a nonsignificant excess risk for cancers of the liver and gall bladder ducts. When the analysis was done for specific lifetime industries, railroad workers had an OR of 1.40 and truck drivers an OR of 1.34 for lung cancer for males. Both these excesses were statistically nonsignificant. Other major findings of interest were an excess of leukemia and multiple myeloma among sales personnel of both sexes and an excess of women with lymphomas and Hodgkin's disease among the health care industry. Cancer of the rectum in both men and women was associated with the retail trade, and cancer of the prostate was more common among ministers, farmers, plumbers, rubber workers, and coal miners.

The several strengths of the TNCS interview data set are its large size, histological confirmation of nearly 95 percent of diagnoses, available information of occupation, and details of confounding variables obtained by personal interview and ability to control for them. Among its weaknesses are a 47 percent nonresponse rate and the fact that the population surveyed came from predominantly urban areas which did not represent many industries. Also, most of the associations observed did not achieve statistical significance because they were based on small numbers of patients who had both specific cancers and specific types of employment. Further, when multiple comparisons are made, some significant associations arise by chance. The authors note that these results should only be used as a research source for hypothesis generation and follow-up studies. This analysis does suggest an association with lung cancer for three industries with potential diesel exhaust exposure. These were trucking, railroading, and mining.

9.3.2. Hall and Wynder (1984): A Case-Control Study of Diesel Exhaust Exposure and Lung Cancer

Hall and Wynder conducted a case-control study of 502 male lung cancer cases and 502 controls without tobacco-related diseases that examined an association between occupational diesel exhaust exposure and lung cancer. Histologically confirmed primary lung cancer patients who were 20 to 80 yr old were ascertained from 18 participating hospitals in 6 U.S. cities, 12 mo prior to the interview. Eligible controls comprised of patients at the same hospitals without tobacco-related diseases and were matched by age (±5 yr), race, hospital, and hospital room status. Five hundred two male lung cancer subjects (64 percent of those who met the study criteria for eligibility) were interviewed. Of the remaining 36 percent, 8 percent refused, 21 percent were too ill or had died, and 7 percent were unreliable. Seventy-five percent of eligible controls completed interviews. Of these interviewed controls, 49.9 percent were from the all cancers category, while 50.1 percent were from the all-noncancers category. All interviews were obtained in hospitals to gather detailed information on smoking history, coffee consumption, artificial sweetener use, residential history, and abbreviated medical history as well as standard demographic variables. Occupational information was elicited by a question on the usual lifetime

occupation and was coded by the abbreviated list of the U.S. Bureau of Census Codes. The odds ratios were calculated to evaluate the association between diesel exhaust exposure and risk of lung cancer incidence. Summary odds ratios were computed by the Mantel-Haenszel method after adjusting for potential confounding by age, smoking, and socioeconomic class. Two-sided, 95 percent confidence intervals were computed by Woolf's method. Occupational exposure to diesel exhaust was defined by two criteria. First, occupational titles were coded "probably high exposure" as defined by the industrial hygiene standards established for the various jobs. The job titles included under this category were warehousemen, bus and truck drivers, railroad workers, and heavy equipment operators and repairmen. The second method used to analyze occupations by diesel exposure used the National Institute for Occupational Safety and Health (NIOSH) criteria. In this method, the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20 percent of their employees exposed to diesel exhaust were defined as having "high exposure". Those with 10 to 19 percent of their employees exposed were defined as having "moderate exposure" and those with less than 10 percent of their empolyees exposed as "low exposure".

Cases and controls were compared with respect to exposure. The odds ratio was 2.0 (95 percent CI = 1.2, 3.2) for those workers who were exposed to diesel exhaust versus those who were not. The risk, however, decreased to a nonsignificant 1.4 when the data were adjusted for smoking. Analyses by NIOSH criteria found a nonsignificant relative risk of 1.7 in the highest probable exposure. There were no significantly increased cancer risks by occupation either by the first method or by the NIOSH method. In order to assess any possible synergism between diesel exhaust exposure and smoking, the lung cancer risks were calculated for different smoking categories. No synergistic effects were observed.

The major strength of this study lies in the availability of detailed smoking history. However, this is more than offset by the lack of diesel exhaust exposure measurements, lack of consideration of latency period, and use of poor surrogate for exposure. Information was collected on only one major lifetime occupation, and it is likely that those workers who had

more than one major job may not have reported the occupation with the heaviest diesel exhaust exposures. Occupational histories were obtained from self report and were not validated with work records. This could have resulted in recall bias and misclassification of exposure status.

9.3.3 Damber and Larsson (1987): Occupation and Male Lung Cancer: A Case-Control Study in Northern Sweden

A case-control study of lung cancer was conducted in northern Sweden to determine the occupational risk factors that could explain the large geographic variations of lung cancer incidence in that country. The study region comprised the three northern most counties of Sweden with a total male population of about 390,000. The rural municipalities with 15 to 20 percent of the total population have forestry and agriculture as dominating industries and the urban areas have a variety of industrial activities (mines, smelters, steel factories, paper mills, and mechanical workshops). All male cases of lung cancer reported to the Swedish Cancer Registry during the 6-yr period between 1972 and 1977 who had died before the start of the study, were selected. Of 604 eligible cases, 5 did not have microscopic confirmation and in another 5 the diagnosis was doubtful, but these cases were included nevertheless. Cases were classified as small carcinomas, squamous cell carcinomas, adenocarcinomas, and other types. For each case a dead control was drawn from the National Death Registry matched by sex, year of death, age, and municipality. Deaths in controls classified as lung cancer and suicides were excluded. A living control matched to the case by sex, year of birth, and municipality was also drawn from the National Population Registry. Postal questionnaires were sent to close relatives of cases and dead controls, and to living controls themselves to collect data on occupation, employment, and smoking habits. Replies were received from 589 cases (98 percent), 582 dead controls (96 percent), and 453 living controls (97 percent).

Occupational data were collected on occupations or employment held for at least 1 yr and included type of industry, company name, task, and duration of employment. Supplementary telephone interviews were performed if occupational data were lacking for any period between age 20 and time of diagnosis. Data analysis involved calculation of the

odds ratio by the exact method based on the hypergeometric distribution and the use of a linear logistic regression model to adjust for the potential confounding effects of smoking. Separate analyses were performed with dead and living controls, and on the whole there was good agreement between the two control groups. A person who had been active for at least 1 yr in a specific occupation was in the analysis assigned to this occupation.

Using dead controls, the odds ratios adjusted for smoking were 1.0 (95 percent CI = 0.9, 1.9) and 2.7 (95 percent CI = 1.2, 6.0) for professional drivers (>1 yr employment), and for underground miners (>1 yr employment) respectively. For 20 or more years of employment in those occupations, the odds ratios adjusted for smoking were 1.2 (95 percent CI = 0.9, 2.6) and 2.7 (95 percent CI = 1.2, 6.0). These were the only two occupations listed with potential diesel exhaust exposure. An excess significant risk was detected for copper smelter workers, plumbers and electricians, as well as concrete and asphalt workers. Occupational asbestos exposure was also associated with an elevated odds ratio of 2.6 (95 percent CI = 1.6, 3.6). All the odds ratios were calculated by adjusting for age, smoking, and municipality.

This study did not detect any excess risk of lung cancer for professional drivers who, amongst all the other occupations listed, have the most potential for exposure to motor vehicle exhaust. However, we do not know if these drivers were exposed exclusively to gasoline exhaust, diesel exhaust, or varying degrees of both. An excess risk was detected for underground miners but it is not known if this is due to diesel emissions from engines or from radon daughters in poorly ventilated mines. Although a high response rate (98 percent) was obtained for the postal questionnaires, the use of surrogate respondents is known to lead to misclassification errors that can bias the odds ratio to 1.

9.3.4. Lerchen et al. (1987): Lung Cancer and Occupation in New Mexico

This is a population-based case-control study conducted in New Mexico which examines the association between occupation and occurrence of lung cancer in Hispanic and non hispanic whites. Cases involved residents of New Mexico, age 25 through 84 yr diagnosed between January 1, 1980, and December 31, 1982, with primary lung cancer, excluding bronchioalveolar carcinoma. Cases were ascertained through the New Mexico

Tumor Registry which is a member of the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute. Controls were chosen by randomly selecting residential telephone numbers and for those over 65 years of age, from the Health Care Financing Administration's roster of Medicare participants. They were frequency-matched to cases for sex, ethnicity, and 10 yr age category with a ratio of 1.5 controls per case. The 506 cases (333 males and 173 females) and 771 controls (499 males and 272 females) were interviewed with a nonresponse rate of 11 percent. Next of kin provided interviews for 50 percent and 45 percent for male and female cases respectively, while for controls the corresponding interviews were provided by 2 percent for each sex. Data were collected by personal interviews conducted by bilingual interviewers in the participants' homes. A lifetime occupational history and a self reported history of exposure to specific agents were obtained for each job held for at least 6 mo from age 12. Questions were asked about the title of the position, duties performed, location and nature of industry, and time at each job title. A detailed smoking history was also obtained. The variables on occupational exposures were coded according to the Standard Industrial Classification scheme by a single person and reviewed by another. To test the hypothesis about the high-risk jobs for lung cancer, *a priori* listing of suspected occupations and industries was created by a two-step process involving a literature review for implicated industries and occupations by the principal investigator. The appropriate Standard Industrial Classification and Standard Occupational Codes associated with job titles were also determined by the principal investigator. For four agents — asbestos, wood dust, diesel exhaust, and formaldehyde — the industries and occupations determined to have exposure were identified, and linking of specific industries and occupations was based on literature review and consultation with local industrial hygienists.

The relative odds were calculated for suspect occupations and industries classifying individuals as ever employed for at least 1 yr in an industry or occupation and defining the reference group as those subjects never employed in that particular industry or occupation. Multiple logistic regression models were used to control simultaneously for age, ethnicity, and smoking status. For occupations with potential diesel exhaust exposure the analysis showed no excess risks for diesel engine mechanics and auto mechanics. Similarly, when

analysed by exposure to specific agents, the odds ratio adjusted for age, smoking and ethnicity was not elevated for diesel exhaust fumes (OR = 0.6, 95 percent CI = 0.2, 1.6). Elevated odds ratios were found for uranium miners (OR = 2.8, 95 percent CI = 1.0, 7.7), underground mining (OR = 2.4, 95 percent CI = 1.2, 4.4), for construction painters (OR = 2.4, 95 percent CI = 0.6, 9.6) and welders (OR = 4.3, 95 percent CI = 1.6, 11.0). No excess risks were detected for the following industries: shipbuilding, petroleum refining, construction, printing, blast furnace, and steel mills, and for the following occupations; construction workers, painters, plumbers, paving equipment operators, roofers, engineers and firemen, woodworkers, and shipyard workers. Females were excluded from detailed analysis because none of the Hispanic female controls had been employed in high-risk jobs; among the non-Hispanic white controls, employment in a high-risk job was recorded for at least five controls for only two industries, construction and painting, for which the odds ratios were not significantly elevated. Data on 333 male cases and 499 controls were therefore analysed.

Among the many strengths of this study are its population-based design, high participation rate, detailed smoking history, and the separate analysis done for the two ethnic groups, southwestern Hispanic and non-Hispanic white males. The major limitations pertain to the occupational exposure date. Job titles obtained from occupational histories were used as proxy for exposure status, but these were not validated. Further, for nearly half the cases, next of kin provided occupational histories. The authors acknowledge the above sources of bias but state without substantiation that these biases would not strongly affect their results. They also did not use a job exposure matrix to link occupations to exposures and do not provide details on the method they used to classify individuals as diesel exhaust-exposed based on reported occupations. The observed absence of an association for exposure to asbestos, a well-established lung carcinogen, may be explained by the misclassification errors in exposure status or by sample size constraints (not enough power). Likewise the association for diesel exhaust reported by only 7 cases and 17 controls also may have gone undetected because of low power. In conclusion, there is insufficient evidence from this study to confirm or refute an association between lung cancer and diesel exhaust exposure.

9.3.5. Garshick et al. (1987): A Case-Control Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers

An earlier pilot study of the mortality of railroad workers by the same investigators (Schenker et al., 1984) found a moderately high risk of lung cancer among the workers who were exposed to diesel exhaust as compared to those who were not. This study was designed to evaluate the feasibility of conducting a large retrospective cohort study. On the basis of these findings the investigators conducted a case-control study of lung cancer in the same population. The population base for this case-control study of lung cancer and diesel exhaust was approximately 650,000 active and retired male U.S. railroad workers with 10 yr or more of railroad service who were born in 1900 or after. The U.S. Railroad Retirement Board (RRB), which operates the retirement system, is separate from the Social Security System and to qualify for the retirement or survivor benefits the workers had to acquire the service of 10 yr or more. Information on deaths that occurred between March 1, 1981, and February 28, 1982, was obtained from RRB. For 75 percent of the deceased population death certificates were obtained from RRB and for the remaining 25 percent they were obtained from the appropriate state department of health. Cause of death was coded according to the ICD-8. The cases were selected from deaths with primary lung cancer, which was the underlying cause of death in most cases. Each case was matched to two deceased controls whose dates of births were within 2.5 yr of the date of birth of the case and whose dates of deaths were within 31 d of the date of death noted in cases. Controls then were selected randomly from workers who did not have cancer noted anywhere on their death certificates and who did not die of suicide or of accidental or unknown causes.

Each subject's work history was determined from a yearly job report filed by his employer with RRB from 1959 until death or retirement. The year 1959 was chosen as the effective start of diesel exhaust exposure for this study, since by this time 95 percent of the locomotives in the United States were diesel powered. Investigators acknowledge that since the transition to diesel powered engines took place in the early 1950s there were some workers who had additional exposure prior to 1959; however, if a worker had died or retired prior to 1959 he was considered unexposed. Exposure to diesel exhaust was considered to be dichotomous for this study; which was assigned based on an industrial hygiene evaluation

of jobs and work areas. Selected jobs with and without regular diesel exhaust exposure were identified by a review of job title and duties. Personal exposure was assessed in 39 job categories representative of workers with and without diesel exhaust exposure. Those jobs for which no personal sampling was done were considered exposed or unexposed on the basis of similarities in job activities and work locations and by degree of contact with diesel equipment. Asbestos exposure was categorized on the basis of jobs held in 1959 or on the last job held if the subject retired before 1959. Asbestos exposure in railroads occurred primarily during the steam engine era and was related mostly to the repair of locomotive steam boilers that were insulated with asbestos. Smoking history information was obtained from the next of kin.

Death certificates were obtained for approximately 87 percent of the 15,059 deaths reported by RRB from which 1374 cases of lung cancer were identified. Fifty-five cases of lung cancer were excluded from the study for either incomplete data (20) or refusal of permission by two states to use information on death certificates to contact the next of kin. Successful matching to at least 1 control with work histories was achieved for 335 (96 percent) cases ≤64 yr of age at death and 921 (95 percent) cases ≥65 yr of age at death. In both age groups 90 percent of the cases were matched with 2 controls. There were 2385 controls in the study, 98 percent were matched within ±31 days of the date of death while the remaining 2 percent were matched within 100 days. Deaths from diseases of the circulatory system predominated among controls. Among the younger workers approximately 60 percent had exposure to diesel while among older workers only 47 percent were exposed to diesel.

Analysis by a regression model, in which years of diesel exhaust exposure was the sum total of the number of years in diesel-exposed jobs, used as a continuous exposure variable, yielded an OR of lung cancer to be 1.39 (95 percent CI = 1.05, 1.83) for over 20 yr of diesel exaust exposure in the younger age group. After adjustment for asbestos exposure and lifetime smoking (pack-years), the OR was 1.41 (95 percent CI = 1.06, 1.88). Increasing years of diesel exhaust exposure categorized as ≥20 diesel years and 5 to 19 diesel-years with 0 to 4 yr as the referent group showed significantly increased risk in the younger age group after adjusting for asbestos exposure and pack-year category of smoking for individuals who

had ≥20 yr of diesel exhaust exposure (OR = 1.64; 95 percent CI = 1.18, 2.29). Subjects with 5 to 19 diesel years had a relative odds of 1.02 (95 percent CI = 0.72, 1.45). In the older age group, only 3 percent of the workers were exposed to diesel exhaust for more than 20 yr. Relative odds for 5 to 19 yr and ≥20 yr of diesel exposure were less than 1 (p >0.01), after adjusting for smoking and asbestos exposure.

Alternate models to explain post asbestos exposure were tested. These were variables for regular and intermittent exposure groups and an estimate of years of exposure based on estimated years worked prior to 1959. No difference in results were seen. The interaction between diesel exhaust exposure and the three pack-year categories <50, >50, and missing pack-years, were explored. The cross-product terms were not significant. A model was also tested that excluded recent diesel exhaust exposure occurring within the 5 yr before death and gave an odds ratio of 1.43 (95 percent CI = 1.06, 1.94) adjusted for cigarette smoking and asbestos exposure.

The results of this study support the hypothesis that occupational exposure to diesel exhaust increases lung cancer risk. The relative odds obtained for this association after adjusting for smoking and asbestos exposure are low, less than 1.5 for the highest exposure category, but the 95 percent confidence interval is narrow.

The many strengths of the study are consideration of confounding factors such as asbestos exposure and smoking; classification of diesel exhaust exposures by job titles and industrial hygiene sampling; exploration of interactions between smoking, asbestos exposure, and diesel exhaust exposure; and high ascertainment (87 percent) of death certificates from the 15,059 deaths reported by the RRB.

The investigators also recognized and reported the following limitations: overestimation of cigarette consumption by surrogate respondents that may have exaggerated the contribution of smoking to lung cancer risk, and use of the ICC job classification as a surrogate for exposure which may have lead to misclassification of diesel exhaust exposure jobs with low intensity and intermittent exposure, such as railroad police, and bus drivers, as unexposed. These two limitations would result in the underestimation of the lung cancer risk. This source of error could have been avoided if diesel exhaust exposures were categorized by a specific dose associated with a job title which could have been classified as

heavy, medium, low, and zero exposure instead of a dichotomous variable. The use of death certificates to identify cases and controls may have resulted in misclassification. Controls may have had undiagnosed primary lung cancer, and lung cancer cases might have been secondary lesions misdiagnosed as primary lung cancer. However, the investigators quote a third National Cancer Survey report in which the death certificates for lung cancer were coded appropriately in 95 percent of the cases. Lastly, as in all previous studies, there is lack of data on the contribution of unknown occupational or environmental exposures and passive smoking. In conclusion, this study, compared with previous studies (on diesel exposure and lung cancer risk), provides the most valid evidence that occupational diesel exhaust emission exposure increases the risk of lung cancer.

9.3.6. Benhamou et al. (1988): Occupational Risk Factors of Lung Cancer in a French Case-Control Study

This is a case-control study of 1625 histologically confirmed cases of lung cancer and 3091 matched controls, conducted in France between 1976 and 1980. This study was part of an international study to investigate the role of smoking and lung cancer. Each case was matched with one or two controls whose diseases were not related to tobacco use, sex, age at diagnosis (±5 yr), hospital of admission, or interviewer. Information was obtained from both cases and controls on place of residence since birth, educational level, smoking, and drinking habits. A complete occupational history was obtained by asking participants to give their occupations from the most recent to the first. Women were excluded because most of them had no occupation. Men who smoked cigars and pipes were excluded because there were very few in this category. Thus the study was restricted to nonsmokers and cigarette smokers. Cigarette smoking exposure was defined by age at the first cigarette (nonsmokers, ≤20 yr, or >20 yr), daily consumption of cigarettes (nonsmokers, <20 cigarettes a day, and ≥20 cigarettes a day), and duration of cigarette smoking (nonsmokers, <35 yr, and ≥35 yr). The data on occupations were coded by a panel of experts according to their own chemical or physical exposure determination. Occupations were recorded blindly using the International Standard Classification of Occupations. Data on 1260 cases and 2084 controls

were available for analysis. Of these, 365 cases and 1007 controls were excluded because they did not satisfy the required smoking status criteria.

A matched logistic regression analysis was performed to estimate the effect of each occupational exposure after adjusting for cigarette status. Matched relative risk ratios were calculated for each occupation with the baseline category, which consisted of patients who had never been engaged in that particular occupation. The matched relative risk ratios adjusted for cigarette smoking for the major groups of occupations showed that the risks were significantly higher for production and related workers, transport equipment operators, and laborers (RR = 1.24, 95 percent CI = 1.04, 1.47). For occupations with potential diesel emission exposure a borderline significant excess risk was found for motor vehicle drivers (RR = 1.42, 95 percent CI = 1.07, 1.89) and transport equipment operators (RR = 1.35, 95 percent CI = 1.05, 1.75). No interaction with smoking status was found in any of the occupations. The only other significant excess was observed for mines and quarrymen (RR = 2.14, 95 percent CI = 1.07, 4.31). None of the significant associations showed a dose-response relationship with duration of exposure.

This study was designed primarily to investigate the relationship between smoking (not occupations and environmental exposures) and lung cancer. While an attempt was made to obtain complete occupational histories, the authors did not clarify if in the logistic regression analysis, they used the subjects first occupation, predominant occupation, last occupation, or ever worked in that occupation as the risk factor of interest. The most important limitation of this study is that the occupations were not coded into exposures for different chemical and physical agents thus precluding the calculation of relative risks for diesel exposure. Using occupations as surrogate measures of diesel exposure, an excess significant risk was obtained for motor vehicle drivers and transport equipment operators, but not for motor mechanics. However, it is not known if subjects in these occupations worked with diesel engines or nondiesel engines.

9.3.7. Hayes et al. (1989): Lung Cancer in Motor Exhaust-Related (MER) Occupations

This study reports the findings from an analysis of pooled data from three lung cancer case-control studies that examine in detail the association between employment in motor exhaust related occupations and lung cancer risk adjusted for confounding by smoking and other risk factors. The three studies were carried out by the National Cancer Institute in Florida (1976 - 79), New Jersey (1980 - 81) and Louisiana (1979 - 83). These three studies were selected because the combined group would provide a sufficient sample to detect a risk of lung cancer in excess of 50 percent among workers in motor exhaust-related occupations. The analyses were restricted to males who had given occupational history. The Florida study was hospital-based with cases ascertained through death certificates. Controls were randomly selected from hospital records and death certificates, excluding psychiatric diseases matched by age and county. The New Jersey study was population-based with cases ascertained through hospital records, cancer registry, and death certificates. Controls were selected from among the pool of New Jersey licensed drivers and death certificates. The Louisiana study was hospital-based (it is not specified how the cases were ascertained), and controls were randomly selected from hospital patients, excluding lung diseases and tobacco related cancers.

A total of 2291 cases of male lung cancers and 2570 controls were eligible, and the data on occupations were collected by next-of-kin interviews for all jobs held for 6 mo or more, including the industry, occupation, and number of years employed. The proportion of next-of-kin interviews varied by site between 50 percent in Louisiana to 85 percent in Florida. The coding schemes were reviewed to identify motor exhaust-related occupations, which included truck drivers, heavy equipment operators (cranes, bulldozers, and graders); bus drivers, taxi drivers, chauffeurs, and other motor vehicle drivers; and automobile and truck mechanics. Truck drivers were classified as routemen and delivery men and other truck drivers. All jobs were also classified with respect to potential exposure to known and suspected lung carcinogens. Odds ratios were calculated by the maximum likelihood method

adjusting for age by birth year, usual amount smoked, and study area. Logistic regression models were used to examine the interrelationship of multiple variables.

A statistically significant excess risk was detected for employment of 10 yr or more for all motor exhaust-related occupations (except truck drivers) adjusted for birth cohort, usual daily cigarette use, and study area. The odds ratio for lung cancer using data gathered by direct interviews was 1.4 (95 percent CI = 1.1, 2.0), allowing for multiple motor exhause, related (MER) employment and 2.0 (95 percent CI = 1.3, 3.0) excluding individuals with multiple MER employment. Odds ratios for all MER employment except truck drivers who were employed for less than 10 yr, were 1.3 (95 percent CI = 1.0, 1.7) and 1.3 (95 percent CI = 0.9, 1.8) for including and excluding multiple MER employment, respectively. No association was observed with employment of less than 10 yr in these occupations. Odds ratios were then derived for specific MER occupations, and, to avoid the confounding effects of multiple MER job classifications, analyses were also done excluding subjects with multiple MER job exposures. Truck drivers employed for more than 10 yr had an odds ratio of 1.5 (95 percent CI = 1.1, 1.9). A similar figure was obtained excluding subjects with multiple MER employment. An excess risk was not detected for truck drivers employed less than ten yr. The only other job category which showed statistically significant excess for lung cancer was the one that included taxi drivers and chauffeurs who worked multiple MER jobs for less than 10 yr (OR = 2.5, 95 percent CI - 1.4, 4.8). For the same category the risk for individuals working in that job for more than 10 yr was 1.2 (95 percent CI = 0.5, 2.6). A statistical significant trend (p <0.05) <2 yr, 2, 9 yr, 10 to 19 yr, and 20+ yr of employment was observed for truck drivers but not for other MER occupations. A non-statistically significant excess risk was also observed for heavy equipment operators, bus drivers, taxi drivers and chauffeurs, and mechanics employed for 10 yr or more. All the above-mentioned odds ratios were derived adjusted for birth cohort, usual daily cigarette use, and state. Exposure to other occupational suspect lung carcinogens did not account for the excess risks detected.

Results of this large study provide evidence that workers in motor exhaust related jobs are at an excess risk of lung cancer that is not explained by their smoking habits or exposures to other lung cancers. Since no information on type of engine use had been

collected, it was not possible to determine if the excess risk was due to diesel exhaust or gasoline exhaust exposure or the mixture of the two. Among its limitations are possible bias due to misclassification of jobs reported by the large proportion of next-of-kin interviews and the problems in classifying individuals into uniform occupational groups based on the pooled data in the three studies, which used different occupational classification schemes.

9.4. CASE-CONTROL STUDIES OF BLADDER CANCER

9.4.1. Howe et al. (1980): Tobacco Use, Occupation, Coffee, Various Nutrients, and Bladder Cancer

This is a Canadian population-based case-control study conducted in the provinces of British Columbia, Newfoundland, and Nova Scotia. These areas were selected because they had cancer registries and were believed not to have concentrations of high-risk industries. All patients with newly diagnosed bladder cancer occurring in the three provinces between April 1974 and June 1976 were identified, and 77 percent of them were interviewed at home. A total of 480 male case-control pairs and 152 female case-control pairs were available for analysis. For each case one neighborhood control, matched by age (±5 yr) and sex, was also interviewed at home to obtain data on smoking, occupation, dietary sources of nitrites and nitrates which convert to nitrosamines (non-public water supply and preserved meat products), and beverage consumption, including a detailed history of coffee consumption. A detailed smoking history was obtained. The occupational history included a chronologic account of all jobs and the number of years and months during which the respondent had worked in each job, experience in industries that were suspected *a priori* to increase the risk of bladder cancer, and exposure to any jobs that involved exposure to dust and fumes at the work place. Relative risk estimates were computed using the linear logistic model applied to individually matched case-control pairs.

A base-line comparison of cases and controls showed that while male patients were similar to controls on income and education, there was an excess of female cases with low family incomes and low levels of educational attainment. For both sexes the mean ages for cases and controls did not differ and the times required for the interview were similar. An

analysis by the *a priori* suspect industries showed elevated risks for a number of industries for males. These included chemical (RR = 7.5, 95 percent CI = 1.7, 67.6), rubber (RR = 5.0, 95 percent CI = 0.6, 236.5), petroleum (RR = 5.3, 95 percent CI = 1.5, 28.6), medicine (RR = 2.6, 95 percent CI = 0.9, 9.3), and the spray painting industry (RR = 1.8, 95 percent CI = 0.7, 4.6). The excess risks were statistically significant only for the petroleum and chemical industry. The estimates did not change when the analysis was done separately for subjects who reported only one exposure and for those who reported exposure to more than one suspect industry. The estimates also remained unchanged after controlling for smoking. Too few females reported working in the *a priori* suspect industries to make any meaningful contribution to the analysis. A statistically nonsignificant excess risk was observed for tanning, electric cable, photographic, commercial paint, tailoring, medicine, food processing, and agricultural industries. The analysis by exposure to dust and fumes in occupations other than those in the a *priori* suspect list detected the relative risks for diesel and traffic fumes (RR = 2.8, 95 percent CI = 0.8, 11.8). Statistically significant excess risks were observed for railroad workers (RR = 9.0, 95 percent CI = 1.2, 394.5) and welding (RR = 2.8, 95 percent CI = 1.1, 8.8). For occupations other than those on the *a priori* list for males and females, statistically significant excesses were detected for metal machinists (RR = 2.7, 95 percent CI = 1.1, 7.6), metal recorders (RR = 2.6, 95 percent CI = 1.0, 7.3), and nursery men (RR = 5.5, 95 percent CI = 1.2, 51.1). Statistically nonsignificant excesses were also detected for exposure to two chemicals: benzidine and its salts, RR=1.3, and bischloromethyl ether, RR = 5.0. A detailed analysis was done for cigarette smoking, which demonstrated statistically significant increasing bladder cancer risk with increasing duration of smoking, total lifetime consumption of packs, and average frequency of cigarettes per day. In males the highest significant risk was observed for latency of less than 35 yr. Then the risk reduced slightly with increasing latency. In females the highest significant risk was for more than 35 yr of latency. Risks were elevated for males consuming all types of coffee and for females consuming instant coffee. Hair dye usage in females and phenacetin usage in males and females carried no risk. Significant risks for use of artificial sweeteners and use of nonpublic water supplies (nitrate and nitrite) were found among males only.

This study was mainly designed to evaluate the various risk factors for bladder cancer such as smoking, coffee consumption, nitrates and nitrites in diet, etc. The major limitation of this study, as the authors noted, was that the three selected provinces did not have high concentrations of industries suspected to be linked to bladder cancer. An excess risk was, however, detected for railroad workers and for those in the "exposed to diesel and traffic fumes category." Risks for those exposed to "diesel fumes only" were not available nor do we know the exact job title of the railroad workers and the type of engines they were operating. The authors also did not detail the method by which they coded the information given by respondents in response to questions on exposure to dust and fumes into the various categories they used in the analysis. These analyses were done for subjects reported having "ever been" exposed vs. "never been exposed" to these fumes, and, although detailed chronological work histories were obtained, no attempt was made to develop a lifetime cumulative exposure index to diesel fumes. In multiple logistic regression models authors used the *a priori* high-risk occupations, hence nothing can be concluded about exposure to diesel exhaust for occupations that were not a part of that list. The authors provide no explanation on possible selection bias as only 77 percent of the eligible population was included in the study. The nonstatistically significant excesses observed for exposure to diesel and traffic fumes suggest the association that warrants further study.

9.4.2. Wynder et. al. (1985): A Case-Control Study of Diesel Exhaust Exposure and Bladder Cancer

A case-control study of diesel exhaust exposure and bladder cancer risk was conducted by Wynder et al. (1985). Cases and controls were obtained from 18 hospitals located in 6 U.S. cities between January 1981 and May 1983. Cases were individuals with histologically confirmed primary cancer of the bladder, diagnosed within 12 mo prior to the interview. Controls were individuals with non-tobacco-related diseases and matched to the case by age (within 8 yr), race, year of interview, and hospital of admission. Women were excluded from the study since the focus was on male-dominated occupations. A structured questionnaire was administered in the hospital to cases and controls to elicit information on

usual occupation, smoking history, alcohol and coffee consumption, as well as other demographic factors.

Two methods were used to define occupational exposure to diesel exhaust. First, occupational titles defined by the industrial hygiene standards as probable high exposure were classified as exposed or not exposed to diesel exhaust. The probable high exposure category consisted of bus and truck drivers, heavy equipment repairmen and operators, railroad workers, and warehousemen. In the second method, guidelines set by NIOSH were used to classify occupations based on the diesel exhaust exposures. In this method the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20 percent of their employees exposed to diesel exhaust were defined as having "high exposure," those with 10 percent to 19 percent of their employees exposed as having "moderate exposure," and those with less than 10 percent of their empolyees exposed as "low exposure." The odds ratio was used as a measure of association to assess the relationship between bladder cancer and diesel exhaust exposure. The overall participation among those eligible and available for interview was 75 percent and 72 percent in cases and controls, respectively.

One hundred ninety-four bladder cancer cases and 582 controls were examined, and the two groups were found to be comparable by age and education. Except for railroad workers who had relative odds of 2.0 (95 percent CI = 0.34, 11.61), the relative odds were less than 1 for other occupations. None of the occupations with diesel exposure such as truck drivers, railroad workers, and heavy equipment workers were found to be associated with bladder cancer. When the risk was examined using the NIOSH criteria for high, moderate, and low exposure, relative odds were 1.68 and 0.16 for high and moderate, respectively, with low as the referent group, neither was statistically significant. Cases and controls were compared by smoking status. Cases were more likely to be current cigarette smokers than controls. Current smokers of 1 to 20 cigarettes/d had relative odds of 3.64 (95 percent CI = 2.04, 6.49), current smokers of 21+ cigarettes/d had relative odds of 3.51 (95 percent CI = 2.00, 6.19), while ex-smokers had relative odds of 1.72 (95 percent CI = 1.01,

2.92). After controlling for smoking there was no significant increase in the risk of bladder cancer for occupations with diesel exhaust exposure compared to occupation without diesel exhaust exposure. A synergistic effect between the two was also not detected.

There are two major methodologic limitations to this study, both pertaining to exposure classification. First, the use of "usual" occupation may have lead to misclassification of those individuals who had held a previous job with diesel exhaust exposure that was not their usual occupation; this may have resulted in reduced power to detect weak associations. Second, since there was no information on amount or duration of diesel exhaust exposure, no analysis on dose-response relationship could be done. Also, no information was available on other confounding risk factors of bladder cancer such as urinary retention, amphetamine abuse, and smoking within the confined space of a truck cab, all of which are life-style factors specific to the truck driving occupation.

9.4.3. Hoar and Hoover (1985): Truck Driving and Bladder Cancer Mortality in Rural New England

This study investigated the relationship between the occupation of truck driving and bladder cancer mortality in a case-control study in New Hampshire and Vermont. Cases included all white residents of New Hampshire and Vermont who died from bladder cancer ICD-8 between 1975 and 1979. Death certificates were provided by the Vital Records and Health Statistics office of the two states, and the next of kin were traced and interviewed in person. Two types of controls were selected for each case. One control was randomly selected from all other deaths excluding suicides and matched on state, sex, race, age (±2 yr), and year of death. The second control was selected with the additional matching criteria of county of residence. Completed interviews were obtained from 325 (out of 410) next of kin for cases and 673 (out of 923) controls. The odds ratio was calculated to ascertain a measure of association between truck driving and bladder cancer. Since separate analyses of the two control series gave similar results, the two control series were combined. Also, since matched analyses yielded results similar to those provided by the unmatched analyses, results of the unmatched analyses were presented.

Sixteen percent (35) of cases and 12 percent (53) of controls had been employed as truck drivers yielding an OR of 1.5 (95 percent CI = 0.9, 2.6) after adjustment for county of residence and age at death. For New Hampshire, the OR was 1.3 (95 percent CI = 0.7, 2.3) and for Vermont the OR was 1.7 (95 percent CI = 0.8, 3.4). For a large number of subjects the next of kin were unable to give the durations of truck driving, and there was an inconsistent positive association with years of truck driving. Crude relative odds were not altered after adjustment for coffee drinking, cigarette smoking, and education as a surrogate for social class. Little variation in risks was seen when the data were analyzed by the industry in which the men had driven trucks. No relationship was seen between age at which employment as a truck driver started and occurrence of bladder cancer. Analysis by duration of employment as a truck driver and bladder cancer showed a positive trend showing increasing relative odds with increasing duration of employment. The odds ratio was statistically significant for the 5 to 9 yr employment category only (RO = 2.9, 95 percent CI = 1.2, 6.7). Similarly, analysis by calendar year first employed showed a statistically significant odds ratio for 1930 to 1949 (RO = 2.6, 95 percent CI = 1.3, 5.1), while relative odds were not significant if they were employed prior to 1929 or after 1950.

The effects of reported diesel exhaust exposure from fuel or engines in truck driving or other occupations were then analyzed. An OR of 1.8 (95 percent CI = 0.5, 7.0) was derived for those who were exposed to diesel exhaust during their truck driving jobs as compared to an OR of 1.5 (95 percent CI = 0.8, 2.7) for those not reporting diesel exhaust exposure. Although none of the individual ORs in the duration categories (0, 1 to 19 yr, 20 to 29 yr, 30 to 39 yr, and 40+ yr) were statistically significant, analysis by self reported duration of exposure to diesel fuel or engines in any occupation and bladder cancer showed a statistically significant positive trend.

This study has investigated an association between truck driving and bladder cancer and was conducted in an attempt to understand the reasons for the high rates of bladder cancer in a rural area of New Hampshire and Vermont. Although an elevated odds ratio for bladder cancer (not statistically significant) was observed for reported truck driving occupations there was insufficient evidence from this study to conclude that excess risk of bladder cancer was due to exposure to diesel emissions. This is because the excess bladder

cancer risk was observed for all truck drivers irrespective of their exposure to diesel emissions. Also, no information is provided on the confounding effects of other aspects of the road environment such as urinary retention, amphetamine abuse, and concentrated cigarette smoke within the truck cab. Other limitations of this study include the use of next of kin for occupational histories who may either under- or overreport occupations and the use of death certificates with their inherent problems of misclassification.

9.4.4. Iscovich et al. (1987): Tobacco Smoking, Occupational Exposure and Bladder Cancer in Argentina

This is a hospital based case-control study of bladder cancer conducted in La Plata, Argentina, to estimate the risk of bladder cancer associated with different types of tobacco, beverages, and occupational exposures. Bladder cancer is one of the most common cancers among males in the La Plata area.

Cases were selected from patients with a histologically confirmed diagnosis of bladder cancer (transitional, squamous-cell, or nonspecific cell type) admitted to the 10 general hospitals in the greater La Plata area (population in 1980 = 580,000) between March 1983 and December 1985. Cases with true bladder papilloma and individuals who were residents of Greater La Plata <5 yr were excluded. Of the 120 cases who were eligible to participate, 1 died prior to the interview, 2 refused to participate and the remaining 117 cases, representing 60 percent of the incident cases registered in the registry, were interviewed. Two control groups (117 neighborhood and 117 hospital controls) were matched by sex and age (±5 yr). Of one hundred seventeen, 99 were males and 18 were females. Hospital controls were selected from the hospital as the case hospitalized for the first time within 3 mo of diagnosis of the case. Twelve percent of the hospital controls had illnesses known to be associated with tobacco smoking. Neighborhood controls were sampled from among persons living in the same block. The interviewer proceeded north in the street block where the case resided and selected the first control who met the matching criteria. Seven hospital controls could not be interviewed because of their poor physical health and were replaced. Three neighborhood controls refused to participate and were replaced. Cases and hospital controls were interviewed at the hospital and the neighborhood controls at their homes to

collect data on demographic, socio-economic, and medical variables, detailed smoking habits, and alcoholic and other beverages consumed.

The interviews were done by trained interviewers, two physicians, and a social worker. The cases and hospital controls were interviewed in the hospital by the physicians, hence, the interviews could not be conducted "blind." A detailed occupational history was obtained for the three occupations of longest duration and the most recent one. For each job title, the activity of the plant and type of production was also ascertained. Job titles were coded according to the International Labor Union (ILO) 1970 classification. Plant activity and type of production were coded according to the United Nations 1975 classification categories. Information was also collected on exposure to 33 chemical and physical agents, which included confirmed or suspected bladder carcinogens. A detailed history of smoking habits was also obtained, and individuals were categorized as current smokers if they were smoking at present or if they had stopped smoking less than two yr previously. Ex-smokers were those who ceased smoking at least or more than 2 yr previously. For each subject a cumulative lifelong consumption of cigarettes by type was estimated and an average consumption of cigarettes per day was computed.

Relative risks were computed for occupational factors using the unconditional logistic regression method adjusting for age and tobacco smoking. These risks were derived for those who were ever employed in that occupation vs. those who were never employed in that occupation. Socioeconomic status of cases and neighborhood control was similar but there were fewer professionals and more manual workers among hospital controls compared with cases. Occupational variables included job title and type of activity of the plant. Significant excess risks were observed for truck and railroad drivers (RR = 4.31, p <0.002) and oil refinery workers (RR = 6.22, p <0.02). The risk for truck and railroad drivers was reduced after adjusting for smoking while that for oil refinery workers increased after adjusting for smoking. The adjusted RRs were not reported. A positive but nonsignificant association was observed for printers (RR = 2.6, p <0.77).

This study identified smoking and coffee drinking as the major risk factors for bladder cancer in this area. The overall age-adjusted RR in males for current smokers relative to nonsmokers was 7.2 (95 percent CI = 3.0, 20.1) with dose-response relationships observed

for the average daily amount as well as for duration of smoking. A strong dose-response relationship was also observed for coffee drinking in males with a RR of 12 (95 percent CI = 4.3, 33.31) for those drinking more than three cups of coffee per day after adjusting for the effect of smoking. No association was found with use of saccharin in males. No results were presented for females for these risk factors.

This case-control study was conducted primarily to determine the reasons for the high rates of bladder cancer in the La Plata region of Argentina. Only 60 percent of the cases registered in the cancer registry were interviewed, and no information was provided for the remaining 40 percent eligible nonrespondents to determine if the study sample was selectively biased in any way. The sample size of 117 was small to start with. The analysis of males reduced it to 99. While the use of two different types of control groups is a strength of this study, none of the interviews were done "blind," and it appears that the hospital interviews were done by the physicians and the neighborhood interviews were done by the social worker. Job titles were used as surrogates of exposure, but the authors state that although they did attempt to analyze by an exposure index derived from a job exposure matrix (details not provided), they found no difference in exposure between cases and controls. This explanation is ambiguous. The authors also grouped truck and railroad drivers together for reasons not mentioned and did not present separate risk estimates. A table of distribution of cases and controls for selected activities or professions did not state if the data pertain to both sexes or males only, and the text did not clarify that either. The reported significant excess risks for truck and railroad drivers were reduced after adjusting for smoking but it was not known if the statistical significance persisted. No analysis was provided for the data collected in the interviews on exposures to the 33 chemical and physical agents and it was not known if the truck and railroad drivers were operating diesel engines or not. Although rare in the La Plata area, the authors acknowledge the occupations known to be associated with bladder cancer (dye, rubber, leather, and textile workers).

9.4.5. Steenland and Burnett (1987): A Case-Control Study of Bladder Cancer Using City Directories as a Source of Occupational Data

The primary objective of the study was to test the usefulness of city directories as a source of occupational data in epidemiologic studies of illness and occupational exposure. Commercial city directories indicating individual data on occupations and employer are compiled from a door-to-door yearly census of all residents 18 yr old and older. They are available in most medium-size cities in the United States. A unique advantage of city directory data is that they identify specific employers and as the authors suggest may be better than death certificates for rapid, inexpensive, record-based, epidemiologic studies.

A case-control study of male bladder cancer deaths in Hamilton County (including Cincinnati), Ohio, was conducted. This county was selected because it is in an industrialized area with high bladder cancer rates and also because city directories cover approximately 85 percent of the people living in this county. A computerized list of all male bladder cancer deaths (n = 731) and all other male deaths (n = 95,057) with the exclusion of deaths from urinary tract tumors and pneumonia, which occurred between 1960 and 1982 was obtained from the Ohio Department of Vital Statistics. Death certificates had been coded by a nosologist according to the ICD code in use at the time of death. A pool of six controls was created for each case matched on sex, residence in Hamilton County at time of death, year of death, age at death (±5 yr), and race. Two types of analysis were performed, one based on city directory data and the other based on death certificate data. In the former, cases and controls were restricted to individuals who had at least one yearly directory listing with some occupational data. The first two controls from the pool of six who met the requirements were selected. This analysis involved 648 cases (627 cases had 2 controls and 21 cases had only 1 control) and 1275 controls.

The death certificate analysis involved all 731 cancer deaths, with 2 controls per case. In most cases, the same two controls were used in this analysis too. The usual lifetime occupation and industry on the death certificate was abstracted from them. Data on occupation and industry were coded with a 3-digit U.S. census code using the method adopted by the U.S. Bureau of Census. Five percent of the occupational data were recorded

for occupation and industry by a second coder, with a high degree of reproducibility. Odds ratios were calculated for bladder cancer using a Mantel-Haenszel procedure.

The city directory data identified four employers significantly associated with bladder cancer deaths; only one of them was identified by the death certificate data, which provided only lifetime type of industry rather than the name of a specific employer. The industries represented by the four employers were a chemical plant, printing company, valve company, and machinery plant. The city directory data analysis demonstrated significant positive associations for quite a few occupations. The occupations that had at least 10 cases or more were engineers (OR = 3.00, p = 0.01), carpenters (OR = 2.36, p <0.01), tailors (OR = 2.56, p <0.01), and furnace operators (OR = 2.5, p = 0.03). Relative odds were increased significantly with increased duration of employment (≥20 yr) for truck drivers (OR = 12, p = 0.01) and furnace operators (based on 4 cases and no controls, p = 0.05). For occupations in which subjects had ever been employed a significant increase in the relative odds with increased duration of employment was observed for the railroad industry (>20 yr of employment, OR = 2.21, p <0.05). Both truck driving and railroad industry occupations involve diesel emission exposures. The analysis of the death certificate data yielded associations in the same direction for most of the occupations. A check of the validity of city directory data indicated that 77 percent of the listings agreed with the social security earnings report for the employer in any given year. A comparison of city directory and death certificate information on occupations indicated a match for occupation between at least one city directory listing and occupation on death certificate for 68.3 percent of the study subjects.

This study demonstrated that city directories are a relatively inexpensive and rapidly accessible source of occupational data in epidemiologic studies. Its many limitations include the problem in tracing women because of the change from maiden to married name, and the availability of data for only the year of residence in the city. They are superior to death certificates in being able to identify high risk employers in specific geographic sites. While death certificate data reflect usual lifetime occupation, city directories yield data on short-term jobs, some of which may involve critical exposure. Thus, a combination of the two approaches may be most productive in record-based hypothesis-generating studies. In the

context of the mentioned pros and cons of using city directories, this study found an excess risk for bladder cancer among two occupations with potential diesel exposure: truck drivers and railroad workers. Two sources of bias that may have influenced these findings are selection bias arising from the use of deaths instead of incident cases since survival for bladder cancer is high and the absence of data on confounding factors such as smoking, beverage consumption, and medication use.

9.5 SUMMARY

Certain extracts of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic in animals and in humans. Further, animal data are suggestive that diesel exhaust is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods of time. It is presumed that diesel engine production will increase if another fuel crisis requiring alternative fuels should occur in the future, since diesel engines are more fuel efficient. This may result in even more excessive levels of diesel exhaust in the ambient air. Since large working populations are currently exposed to diesel exhaust and since nonoccupational exposures, currently are of concern as well, the possibility that exposure to this complex mixture may be carcinogenic to humans has become an important public health issue.

A major difficulty with the occupational studies considered here was the measurement of the actual diesel exhaust exposure. Most studies compared men in job categories with presumably some exposure to diesel exhaust with either standard populations (presumably no exposure to diesel exhaust) or with men in other job categories from industries with little or no potential for diesel exhaust exposure. A few studies have included measurements of diesel fumes, but there is no standard method for the measurement. No attempt is made to correlate these exposures with the cancers observed in any of these studies, nor is it clear exactly which extract should be measured to assess the occupational exposure to diesel exhaust. The occupations involving potential exposure to diesel exhaust are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators.

With the exception of the study by Waxweiler et al. (1973), there have been no known studies of miners to assess whether diesel exhaust is associated with lung cancer. Virtually all miners (metal and coal) use diesel equipment, which was introduced in the early 1960s. Estimates of how many of these miners are exposed to diesel fumes are not available.

The cohort studies mainly demonstrated an increase of lung cancer. Studies of bus company workers by Waller (1981), Rushton et al. (1983), and Edling et al. (1987) failed to demonstrate any statistically significant excess risk of lung cancer, but these studies have certain methodological problems such as small sample sizes, short follow-up periods, lack of information on confounding variables and lack of analysis by duration of exposure or latency that preclude their use in determining the carcinogenicity of diesel exhaust. Although the Waller (1981) study had a 25-yr follow-up period the cohort was restricted to only employees (ages 45 to 64) currently in service. Employees who left the job earlier, as well as those who were still employed after age 64 and who may have died from cancer, were excluded.

Wong et al.,(1985) conducted a mortality study of heavy equipment operators that demonstrated a significant increased risk of liver cancer in total and in various subcohorts. The same analysis also showed statistically significant deficits in cancers of the large intestine and rectum. Metastasis from the cancers of the large intestine and rectum in the liver probably got misclassified as primary liver cancer which lead to an observed excess risk. This study did demonstrate a nonsignificant positive trend for cancer of the lung with length of membership and latency. Individuals without work histories who started work prior to 1967 when records were not kept may have been the ones who were in the same job for the longest period of time. The workers without job histories included those who had the same job before and after 1967 and thus may have worked about 12 to 14 yr longer; these workers exhibited significant excess risks of lung cancer and stomach cancer. If this assumption about their jobs is correct, then these site- specific causes can be linked to diesel exhaust exposure. However, this study has quite a few methodological limitations such as the absence of detailed work histories for 30 percent of the cohort and the availability of only partial work histories for the remaining 70 percent, thus jobs were classified and ranked according to presumed diesel exposure. Information is lacking regarding duration of

employment in the job categories (used for surrogate of exposure), and other confounding factors (alcohol consumption, cigarette smoking, etc.). Thus, this study can not be used to support a causal association or to refute the same between exposure to diesel exhaust and lung cancer.

A two-yr mortality analysis of the American Cancer Society's prospective study by Boffetta et al. (1988), after controlling for age and smoking, demonstrated an excess risk of lung cancer in certain occupations with potential exposure to diesel exhaust. These excesses were statistically significant among miners (RR = 2.67, 95 percent CI = 1.63 to 4.37) and heavy equipment operators (RR = 2.6, 95 percent CI = 1.12 - 6.06). The elevated risks were nonsignificant in railroad workers (RR = 1.59) and truck drivers (RR = 1.24). A dose response was also observed for the truck drivers. With the exception of miners, exposure to diesel exhaust occurred in the three other occupations showing an increase in the risk of lung cancer. Despite the methodologic limitations such as the lack of representiveness of the study population composed of volunteers only and the questionable reliability of exposure data based on self-administered questionnaires which were not validated, this study is suggestive of a causal association between exposure to diesel exhaust and excess risk of lung cancer.

There were two mortality studies conducted on railroad workers by Howe et al. (1983) in Canada and Garshick et al. (1988) in the United States. The Canadian study found relative risks of 1.2 (p <0.01) and 1.35 (p <0.001) among "possibly" and "probably" exposed groups, respectively. The trend test showed a highly significant dose response relationship with exposure to diesel exhaust and the risk of lung cancer. The main limitation of the study was the inability to separate overlapping exposures of coal dust and diesel fumes. Information on jobs was available at retirement only. There was also insufficient detail on the classification of jobs by diesel exhaust exposure. The exposures could have been nonconcurrent or concurrent, but, since the data are lacking, it is possible that observed excess could be due to the effect of both coal dust and diesel fumes and not due to just one or the other. However, it should be noted that, so far, coal dust has not been demonstrated to be a pulmonary carcinogen in studies on coal miners. But lack of data on

confounders such as asbestos and smoking makes interpretation of this study difficult. The findings of this study are at the best suggestive of diesel exhaust being a lung carcinogen.

The most definitive evidence for linking diesel exhaust exposure to lung cancer comes from a railroad worker study conducted in the United States. Relative risks of 1.57 (95 percent CI = 1.19 to 2.06) and 1.34 (95 percent CI 1.02 to 1.76) were found for ages 40 to 44 and 45 to 49, respectively, after the exclusion of workers exposed to asbestos. This study also found that risk of lung cancer increased with increasing duration of employment. This large cohort study with lengthy follow up and adequate analysis, including dose response (based on duration of employment as a surrogate) as well as adjustment for other confounding factors such as asbestos and smoking, makes the observed association between increased lung cancer and exposure to diesel exhaust more meaningful.

Among the seven lung cancer case-control studies reviewed in this document, the study by Lerchen et al. (1987) did not find increased risk of lung cancer, after adjusting for age and smoking, for diesel fume exposure. The major limitation of this study was lack of adequate exposure data derived from the job titles obtained from occupational histories. Next of kin provided the occupational histories for 50 percent of the cases which were not validated. The power of the study was small (analysis done on males only, 333 cases). On the other hand statistically nonsignificant excess risks were observed for diesel exhaust exposure by Williams et al. (1977) in railroad workers (OR = 1.4) and truck drivers (OR = 1.34), by Hall and Wynder (1984) for workers who were exposed to diesel exhaust versus who were not (OR = 1.4 and 1.7 with two different criteria), and by Damber and Larsson (1987) in professional drivers (OR = 1.2). These rates were adjusted for age and smoking. Both Williams et al. (1977) and Hall and Wynder (1984) had high nonparticipation rates of 47 percent and 36 percent respectively. In addition, the self-reported exposures used in the the study by Hall and Wynder (1984) were not validated. This study also had low power to detect excess risk of lung cancer for specific occupations.

The study by Benhamou et al. (1988), after adjusting for smoking, found significantly increased risks of lung cancer among French motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35). The main limitation of the study was inability to separate the exposures to diesel exhaust from those of gasoline exhausts since both motor

vehicle drivers and transport equipment operators probably were exposed to the exhausts of both types of vehicles. Hayes et al. (1989) combined data from three studies (conducted in three different states) to increase the power to detect an association of lung cancer with different occupations that had high potential for exposure to diesel exhaust. They found that truck drivers employed for more than 10 years had a significantly increased risk of lung cancer (OR = 1.5, 95 percent CI = 1.1 to 1.9). This study also found a significant trend of increasing risk of lung cancer with increasing duration of employment among truck drivers. These relative odds were computed by adjusting for birth cohort, smoking, and state of residence. The main limitation of this study is again the mixed exposures to diesel and gasoline exhausts, since information on type of engine was lacking. Potential bias may have been introduced because the way in which the cause of death was ascertained for the selection of cases varied in the three studies. The methods used in these studies to classify the occupational catagories are different hence probably leading to incompatability of occupational catagories.

The most convincing evidence comes from the Garshick et al. (1987) case-control study among railroad workers. After adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95 percent CI = 1.05 to 1.83). Among the younger workers with longer diesel exhaust exposure, the risk of lung cancer increased with the duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent diesel exhaust exposure (5 yr before death) relative odds increased to 1.43 (95 percent CI = 1.06 - 1.94). This study appears to be well conducted and well- analyzed case-control study with reasonably good power. Potential confounders were controlled adequately, and interactions between diesel exhaust and other lung cancer risk factors were tested.

Of the five bladder cancer case-control studies, three studies found increased risk in occupations with a high potential diesel exhaust exposure. A significantly increased risk of bladder cancer was found in Canadian railroad workers (RR = 9.0, 95 percent CI = 1.2 to 349.5; in Howe et al., 1980) and in Argentinean truck and railroad drivers (RR = 4.31, $p < 0.002$; Iscovich et al., 1987). Significantly increased risks were observed with increasing duration of employment of ≥20 yr in truck drivers (OR = 12, $p = 0.01$) and railroad industry workers (OR = 2.21, $p < 0.05$; Steenland and Burnett, 1987). No significant increased risk

was found for any diesel-related occupations in studies by Wynder et al. (1985) and by Hoar and Hoover et al. (1985). All these studies had several limitations including inadequate characterization of diesel exhaust exposure, lack of validation of surrogate measures of exposure, and presence of other confounding factors (urinary retention, concentrated smoke within the truck cab, etc.); most of them had small sample sizes, and none presented any latency analysis.

In summary, an excess risk of lung cancer was observed in three out of seven cohort studies and six out of seven case-control studies. Of these studies, two cohort and two case-control studies observed a dose-response relationship using duration of employment as a surrogate for dose. However, because of the lack of actual data on exposure to diesel exhaust in these studies and other methodologic limitations such as lack of latency analysis, etc. the evidence of carcinogenicity in humans is considered to be limited for diesel exhaust exposure.

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10. WEIGHT OF EVIDENCE FOR CARCINOGENICITY AND UNIT RISK ESTIMATES

10.1. INTRODUCTION

This chapter provides a quantitative assessment of carcinogenicity risk and calculates the unit risk estimate of diesel engine emissions based on animal inhalation bioassay data. (Unit risk is a characterization of the carcinogenic potency for a compound.) The unit risk estimate for an air pollutant is defined as the increased lifetime cancer risk for an individual who is continuously exposed to an air pollutant at a concentration of 1 $\mu g/m^3$ in ambient air. We are now in the process of acquiring human epidemiological data (Garshick et al., 1987, 1988) to perform cancer risk calculations. The unit risk estimate calculated from these human data may provide a useful comparison with that calculated from animal data.

Animal studies show a definite relationship between exposure to diesel engine emissions and the induction of tumors, but issues remain as to their relevance to humans. Although several inhalation studies of rats (Ishinishi et al., 1986) reported a relationship between exposure to diesel exhaust and induction of lung tumors, these effects have been interpreted (Vostal, 1986) as being produced by "particle overload" due to inhibition of particle clearance from the lungs at high exposure concentrations. However, since diesel exhaust contains many known carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), long-term inhalation of such compounds could induce lung tumors even without particle overload. Nevertheless, the potential effects of particles must be considered. First of all, the site of particle deposition will determine, at least to some extent, the target site. The delivered dose will vary with particle deposition efficiency. The delivered rate of particle absorbed organics will differ from exposure to pure organics. Finally, particle-containing macrophages may release factors capable of acting as either cocarcinogens or promoters.

The exact role of particles in the formation of lung tumors is not well understood. A pure particle overload effect (Morrow, 1988) on the induction of lung tumors occurs in rats only when the animals inhale "nuisance dust"[e.g., titanium dioxide (TiO_2)] at very high concentrations (Lee et al., 1986). The particle burdens in the lung which led to lung tumor formation in the TiO_2-exposed animals were 30 to 60 times higher than those found in the groups in which lung tumors were produced by high diesel exposure. The incidence of lung tumors did not increase when rats had particulate lung burdens of that were 11 times greater than those producing lung tumors from diesel exhaust soot (Table 10-1). Even the volumetric burden was higher by a factor of 11 to 20

TABLE 10-1. PARTICULATE LUNG BURDEN AND LUNG TUMORS IN RATS FOLLOWING A 2-YR EXPOSURE TO TITANIUM DIOXIDE (TiO_2) AND DIESEL EXHAUST (MEAN FOR MALE AND FEMALE RATS)

	Exposure concentration (mg/m^3)	Particle load		Lung tumors percent of rats
		mg/g lung	mm^3/g lung	
TiO_2	0	-	-	2.5
	10	25.5	6.0	2.1
	50	124	29.1	1.3
	250	665	156.1	26[a]
Diesel exhaust	0	-	0.9	
	0.35	0.6	0.4	1.3
	3.5	11.5	- -	3.6[a]
	7.0	20.5	13.7	12.8[a]

[a] Significantly different from controls.

SOURCE: Lee et al., 1985, 1986; Wolff et al., 1987.

(156.1/13.7 to 156.1/7.7) in TiO_2-exposed animals with lung tumors than in tumor-bearing animals exposed to diesel exhaust. (Volumetric burden may be important in the functional impairment of macrophages [Morrow, 1988]). A volumetric lung burden of TiO_2 particles that was almost fourfold higher (29.1/7.7) than in tumor-bearing animals exposed to diesel exhaust did not lead to tumor formation.

This comparison suggests that the particle overload effect on lung tumor induction depends heavily on the presence of organic material absorbed to the particles. That is, the particle load in lungs in the rats of the diesel studies could have synergistically influenced the carcinogenic potency of the inhaled organic compounds associated with particles by increasing lung epithelial cell proliferation rates that are due to the release of mediators from activated inflammatory cell, or by increasing the retention of organic compounds.

Recent results on the formation of benzo[a]pyrene (B[a]P) metabolites and DNA adducts in lungs of rats after exposure to diesel exhaust and to B[a]P adsorbed onto carbon black particles suggest that inhaled organic compounds are related to the initiation of lung

tumors (Wolf et al., 1989). Particles may play an important role in the carcinogenic potential of inhaled diesel exhaust in so far as they prolong the retention of the associated organics. Indeed, early experiments by Saffiotti et al. (1964, 1965) and by Henry and Kaufman (1973) and Henry et al. (1975) indicated that lung tumor incidences were increased when B[a]P was intratracheally instilled adsorbed on particles (Fe_2O_3 or carbon) as opposed to instillation of the pure compound.

When administered alone, the high dose of B[a]P arriving at a target cell over a short period of time may overwhelm the cell's ability to metabolize B[a]P and to form carcinogenic metabolites. In this case, much of the B[a]P is quickly cleared via the blood circulation (Wolff et al., 1989). On the other hand, the slower release of B[a]P (i.e., a slower dose rate delivered to target cells, over a longer period of time in the case of B[a]P administered with particles) may allow more metabolites to form which are responsible for tumor induction.

Results on the formation of B[a]P metabolites after inhalation of the pure compound vs. inhalation of B[a]P plus particles show that the latter led to the formation of more metabolites in the lung (Sun et al., 1984; Bond et al., 1986). In these studies, covalent binding of B[a]P with macromolecules was increased 10- to 20-fold when B[a]P was adsorbed to carbon black compared to inhalation of the pure compound. In addition, prolonged delivery of B[a]P to target cells when inhaled with particles implies that possibly more cells in their sensitive phase can be exposed to the carcinogen, assuming a given cell proliferation rate. This means that the duration of exposure is important in tumor induction. This issue needs further investigation.

Understanding the roles played by particles and organic compounds is essential for biologically based dose-response modeling. In the absence of any scientific information, a reasonable approach for the risk estimate is to assume that particles only prolong the retention of the associated organic compounds. It does not seem appropriate to assume that particles could act as a complete carcinogen. In this report, several approaches, with and without adjusting for the overload effect, are used to calculate unit risk estimates.

Since little is known about the carcinogenic mechanism of inhaled diesel exhaust, we cannot extrapolate with confidence the positive results of the rat inhalation studies to

humans. However, it may indicate the magnitude of risk associated with environmental or occupational exposure of humans to diesel exhaust, assuming that humans respond like rats.

10.2. REVIEW OF PREVIOUS RISK ESTIMATES

Early attempts to quantitatively assess the carcinogenicity of diesel engine emissions were hindered by a lack of well-controlled epidemiologic studies and long-term animal cancer bioassays. In attempts to surmount these obstacles, investigators used the "comparative potency" method (Albert et al., 1983) and an approach that involved estimating risk from a nonpositive epidemiologic study (Harris, 1983).

In the comparative potency method, a combustion product is selected that has a previously determined potency estimate based upon epidemiologic data. The ratios of the potency of this agent (e.g., coke oven emissions) to diesel particulate matter extract in a variety of mutagenicity and carcinogenicity tests are then multiplied by the epidemiology-based potency estimate for coke oven emissions and averaged. If epidemiology-based estimates from more than one pollutant are used, the derived potencies are generally averaged to obtain an overall mean.

The comparative potency estimate of Albert et al. (1983) is probably best known. Their results were obtained using epidemiology-based unit cancer risk estimates for coke oven emissions, cigarette smoke condensate, and roofing tar. Samples of particulate matter were collected from three light duty-engines (a Nissan 220 C, an Oldsmobile 350, and a Volkswagen turbocharged Rabbit), all run on a highway fuel economy test cycle and a heavy duty engine (Caterpillar 3304), run under steady-state, low-load conditions.

The particulate matter was extracted with dichloromethane and tested in a variety of assays. Those selected for development of comparative potency estimates included:

- Ames *Salmonella typhimurium* (TA98) reverse mutation;
- gene mutation in L5178Y mouse lymphoma cells;
- viral enhancement of chemical transformation in Syrian hamster embryo cells; and
- Sencar mouse skin tumor initiation.

The first two studies were carried out in the presence of metabolic activators. The potency, defined as the slope of the dose-response curve, was measured for each sample in each short-term assay.

The skin tumor initiation test gave definitive positive results only for the Nissan engine. Since this was considered to be the most biologically relevant test, it was used to derive potency estimates for the Nissan engine. This was achieved by multiplying the epidemiology-based potency estimates for each of the three agents (coke oven emissions, roofing tar, and cigarette smoke condensate) by the ratios of their potencies in the skin tumor initiation test to that of the Nissan diesel. According to this method, the estimated lifetime cancer risk/$\mu g/m^3$ of extractable organic matter is as follows:

Coke oven emissions	Roofing tar	Cigarette smoke condensate
2.6×10^{-4}	5.2×10^{-4}	5.4×10^{-4}

The average of the three equals 4.4×10^{-4}.

The potency of the other diesel emission samples was not estimated directly because of the weak response in the skin tumor initiation test. Instead, their potency relative to the Nissan engine was estimated as the arithmetic mean of their potency relative to the Nissan in the *Salmonella* assay in strain TA 98, the sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells, and the mutation assay in mouse lymphoma cells. The estimated lifetime cancer risk per $\mu g/m^3$ extract was:

Volkswagen	Oldsmobile	Caterpillar
1.3×10^{-4}	1.2×10^{-4}	6.6×10^{-6}

To convert these values to a lifetime risk per $\mu g/m^3$ of total particulate matter, it was necessary to multiply these results by the fraction of extractable organic matter in the particles. These fractions were:

Nissan	Volkswagen	Oldsmobile	Caterpillar
0.08	0.18	0.17	0.27

After this adjustment, the resulting estimated potencies were equal to 3.5×10^{-5}, 2.3×10^{-5}, 2.0×10^{-5}, and $1.8\times10^{-6}/\mu g/m^3$ of inhaled diesel particulate matter.

Harris (1983) analyzed the data on the four engines used by Albert et al. (1983), but used only two epidemiology-based potency estimates, those for coke oven emissions and roofing tar. He used three of the same assays as did Albert et al. (1983) the Sencar mouse skin tumor initiation assay, enhancement of viral transformation in hamster embryo cells, and the L5178 mouse lymphoma test. The third assay, however, was used both with and without metabolic activation, while the *Salmonella* assay was not used. Harris also obtained slightly different potency values from the bioassays, even though most of the same data sets were used.

The diesel potency estimates were then derived by multiplying the potencies for both coke oven and roofing tar by the ratio of their potencies compared to diesel in each of the four bioassays. For example, the epidemiology-based potency for coke oven emissions was estimated to equal $4.4\times10^{-4}/\mu g/m^3$. In the skin tumor initiation test, 2.1 papillomas per mouse were reported for the coke oven sample, compared with 0.53 for the Nissan engine extract. The benzene extractable fraction was 0.06. The diesel potency estimate using this comparison is then

$$(0.53/2.1)\times0.06\times4.4\times10^{-4}/\mu g/m^3$$

or $6.6\times10^{-6}/\mu g/m^3$ particulate matter. A total of eight comparisons were made for each engine, four bioassays times two epidemiology-based potency estimates.

The Harris estimates are not comparable to those of Albert et al. without adjustment. The unit risk estimates of Albert et al. are based upon absolute risk during lifetime exposure, while Harris reported his values in terms of relative risk per year of exposure. To

adjust this to lifetime extra risk for continuous exposure, it is necessary to multiply Harris's values by a factor of

$$8.2\ [= (24\ h/8\ h)\times 70\times 0.039]\ ,$$

where (24/8) adjusts from the 8-h work day to 24-h continuous exposure, 70 reflects the lifetime exposure (70 yr), and 0.039 is the lifetime lung cancer mortality rate in the U.S. population.

The range of unadjusted potencies included $0.02\text{-}0.06\times10^{-4}$ for the Nissan sample, $0.01\text{-}0.24\times10^{-4}$ for the Oldsmobile 350, $0.02\text{-}2.78\times10^{-4}$ for the Volkswagen Rabbit, and $0.01\text{-}0.25\times10^{-4}/\mu g/m^3$ particulate matter for the Caterpillar sample. Harris (1983) derived an overall mean relative risk value of $3.5\times10^{-5}/\mu g/m^3$ for the three light-duty engines. Individual mean values for each engine were not reported. After conversion to a unit risk, the estimated potency of the particulate matter from the three engines was equal to $2.9\times10^{-4}/\mu g/m^3$.

McClellan (1986), Cuddihy et al. (1981, 1984), and Cuddihy and McClellan (1983) reported a potency factor of about $7.0\times10^{-5}/\mu g/m^3$ using a comparative potency method similar to those reported above. The data base was similar to that used by Albert et al. (1983) and Harris (1983), although there were some differences. In addition to the epidemiology-based potency estimates for coke oven emissions, roofing tar, and cigarette smoke condensate, the authors derived a human potency estimate for urban soot. This estimate was based upon an average air concentration of 0.06 mg/m^3 and an annual cancer incidence of 7/100,000. Interestingly, this is equivalent to a lifetime risk of $8.0\times10^{-5}/\mu g/m^3$, a value very similar to estimates derived for diesel particulate matter.

Harris (1981) also assessed the risk of exposure to diesel engine emissions using data from the London Transport Worker Study reported by Waller (1981). Five groups of employees from the London Transport Authority (LTA) were used. These included bus garage engineers, bus drivers, bus conducters, engineers in central works, motormen, and guards. The first group was considered the highest exposed, the next two intermediate, and the last two unexposed.

When cancer death rates for the high exposure group were compared with those for London males, the observed to expected (O/E) ratios were less than 1. This was thought to be due to a "healthy worker effect." Harris therefore merged the three exposed groups and compared them with the two groups considered to be unexposed. An adjustment was made for the estimated greater exposure levels of the garage engineers as compared to bus drivers and conductors. The relative risk was greater than one but was statistically significant only for garage engineers exposed from 1950 to 1960.

Harris (1983) identified a variety of uncertainties relative to potency assessment based on this study. These included:

- Small unobserved differences in smoking incidences among groups, which could have a significant effect on lung cancer rates.
- Uncertainty about the magnitude of exposure in the exposed groups.
- Uncertainty regarding the extent of change in exposure conditions over time.
- Random effects arising from the stochastic nature of cancer incidence.
- Uncertainty in the mathematical specification of the model.

Taking these uncertainties into account, he derived a maximum likelihood relative risk estimate of 1.23×10^{-4} with a 95-percent upper confidence limit of $5\times10^{-4}/\mu g/m^3$ particulate matter/yr. These estimates are equal to 1.0×10^{-3} and 4.1×10^{-3}, respectively, when converted to an absolute risk for lifetime exposure to 1 $\mu g/m^3$ particulate matter.

The potency estimates derived using various approaches are summarized in Table 10-2. Estimates for the Caterpillar engine were not included, since it was not run under normal use conditions. The largest estimate, $4.1\times10^{-3}/\mu g/m^3$, was derived by Harris (1983), using data from the London Transport Workers study. The smallest estimate, $2.5\times10^{-5}/\mu g/m^3$, is the mean from three light-duty engines evaluated by Albert et al. (1983) using the comparative potency method.

Some of the important potential sources of error associated with the comparative potency method include:

- Uncertainty regarding the composition of roofing tar and coke emissions.
- Omission of the possible effects of gaseous phase emissions are not included in the potency estimates;
- Uncertainty regarding the epidemiology based potency estimates.
- Uncertainty regarding the bioavailabilty of the particle adsorbed organics following extraction with an organic solvent than *in vivo*.
- The assumption that short-term tests define carcinogenicity both quantitatively and qualitatively.

TABLE 10-2.

ESTIMATED LIFETIME RISK OF CANCER FROM INHALATION OF 1 $\mu g/m^3$ DIESEL PARTICULATE MATTER

Basis	Lifetime Risk	Comments	Reference
Comparative potency	3.5x10-5	Nissan engine	Albert et al., 1983
Comparative potency	2.6x10-5	Average of three engines	Albert et al., 1983
Comparative potency	7.0x10-5	Light-duty engines	McClellan et al., 1986
Comparative potency	2.9x10-4	Average of three engines	Harris, 1983
Epidemiology	1.0x10-3	London transport study	Harris, 1983

10.3. RISK CALCULATIONS BASED ON ANIMAL BIOASSAY DATA

10.3.1. Data Available for Risk Calculations

As reviewed in Chapter 7, five bioassay studies showed positive lung tumor response in rats (Brightwell et al., 1986; Ishinishi et al., 1986; Iwai et al., 1986; Mauderly et al., 1987; Stöber, 1986). Only data from three studies (Tables 10-3 to 10-5) are selected for risk calculations because each study consists of multiple exposure groups and thus is more appropriate for risk calculations. The times to event (i.e., death with or without tumors) are available for the Mauderly et al. study. These time-to-event data are used in all risk calculations, except when data from low exposure groups (i.e., groups with the adjusted dose less than 0.73 mg/m^3) from different studies are pooled (Table 10-6). The pooling of the low exposure groups provides a data base for calculating unit risk estimates for which the particle overload effect is minimized to the extent possible without relying on mathematical modeling. The risk estimates calculated from these data serve a useful basis for comparison with ones calculated with more mathematical sophistication.

10.3.2. Calculation of Unit Risks

The linearized multistage model is used to calculate unit risk estimates, using various dose equivalence assumptions. Two approaches are used to calculate unit risks: the first approach considers both the particle overload effect and the amount of particle-associated organics in the lung, while the second approach uses the conventional approach to calculate lung burden without considering either amount of particle associated organics in the lung or the particle overload effect.

TABLE 10-3

INCIDENCE OF LUNG TUMORS IN FISHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO DIESEL EXHAUST IN AIR

Experimental dose (mg/m^3)	Adjusted dose[a]		Organics dose (mg/cm^2)[b]	Lung tumor incidence rate[c]
	mg/m^3	mg/kg/d		
0	0	0	0	2/230
0.35	0.073	0.049	2.5×10^{-6}	3/223
3.47	0.73	0.48	3.6×10^{-5}	8/222
7.08	1.48	0.98	7.3×10^{-5}	29/227

[a]Dose (mg/kg/d) = adjusted dose (mg/m^3) x ($0.2\ m^3$/d)/0.3 kg, where $0.2\ m^3$ is assumed to be the daily air intake volume for a 0.3-kg rat, and where adjusted dose = experimental dose x 7/24 x 5/7, reflecting that animals were exposed only 7 h/d, 5 d/wk for life. These doses are used in the Approach 2 calculations.

[b]They are calculated by using mathematical models and are used in the Approach 1 calculations. cm^2 is a lung surface area.

[c]Time-to-tumor data are used in all risk calculations except for the pooled data calculation.

SOURCE: Mauderly et al., 1987.

TABLE 10-4. INCIDENCE OF LUNG TUMORS IN FISHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO HEAVY-DUTY ENGINE EXHAUST

Experimental dose (mg/m^3)	Adjusted dose[a]		Organics dose (mg/cm^2)[b]	Lung tumor incidence rate[c]
	mg/m^3	mg/kg/d		
0	0	0	0	1/123
0.46	0.26	0.18	9.6×10^{-6}	1/123
0.96	0.55	0.37	2.8×10^{-5}	0/125
1.84	1.05	0.70	5.3×10^{-5}	4/123
3.72	2.13	1.42	1.1×10^{-4}	8/124

[a] Dose (mg/kg/d) = adjusted dose (mg/m^3)x(0.2 m^3/d)/0.3 kg, where 0.2 m^3 is assumed to be the daily air intake volume for a 0.3-kg rat, and where adjusted dose = experimental dose x 16/24 x 6/7, reflecting that animals were exposed only 16 h/d, 6 d/week for life. These doses are used in Approach 2 calculations.

[b] They are calculated by using mathematical models and are used in the Approach 1 calculations.

SOURCE: Isinishi et al., 1986.

TABLE 10-5. INCIDENCE OF LUNG TUMORS IN FISHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO DIESEL EXHAUST IN AIR

Experimental dose (mg/m^3)	Adjusted dose, mg/m^3	Adjusted dose, mg/kg/d	Organics dose $(mg/cm^2)^b$	Lung tumor incidence rate[c]
0	0	0	0	4/282
0.7	0.33	0.22	1.2×10^{-3}	1/144
2.2	1.05	0.70	5.2×10^{-3}	14/144
6.6	3.14	2.10	1.6×10^{-4}	55/143

[a] Dose (mg/kg/d) = adjusted dose (mg/m^3) × ($0.2\ m^3$/d)/0.3 kg, where $0.2\ m^3$ is assumed to be the daily air intake volume for a 0.3-kg rat, and where adjusted dose = experimental dose x 16/24 x 5/7, reflecting that animals were exposed only 16 h/d, 5 d/week for life. These doses are used in the Approach 2 calculations.

[b] They are calculated by using mathematical models and are used in the Approach 1 calculations.

SOURCE: Brightwell et al., 1986.

TABLE 10-6. COMBINED DOSE-RESPONSE DATA FROM FOUR STUDIES CONDUCTED ON FISHER 344 RATS*

Experimental dose (mg/m^3)	Adjusted dose (mg/m^3)	Lung tumors	Remarks/References
0	0	11/773	All control groups
0.11	0.063	3/123	Ishinishi et al.,1986 (light-duty engine)
0.35	0.073	3/223	Mauderly et al, 1987
0.41	0.23	1/125	Ishinishi et al,1986 (light-duty engine)
0.46	0.26	1/123	Ishinishi, et al, 1986 (heavy-duty engine)
0.70	0.33	1/144	Brightwell et al, 1986
0.96	0.55	0/125	Ishinishi et al, 1986 (heavy-duty engine)
1.08	0.62	5/123	Ishinishi, et al, 1986 (light-duty engine)
3.47	0.73	8/221	Mauderly, et al, 1987

* Only those groups with an adjusted dose less than 0.73 mg/m^3 are included in the table.

10.3.2.1. Approach 1

The deposition-clearance-retention model for inhaled diesel particles for the rat (described in Appendix C) takes into account deposition efficiency and a retardation of particle clearance from the alveolar region at high inhaled concentrations due to a particle overload effect. This overload effect accounts for the difference (as shown in Figure 10-1) between high and low exposure concentrations with respect to the organics lung burden (mg) per unit of air concentration. When this approach is used, a retained dose (particles or particle associated organics) per unit of epithelial surface area (or weight) of the lung can be calculated for each exposure situations in different rat studies. In these calculations, the mass fraction of the total absorbed organics in a diesel particle is assumed to be 20 percent,

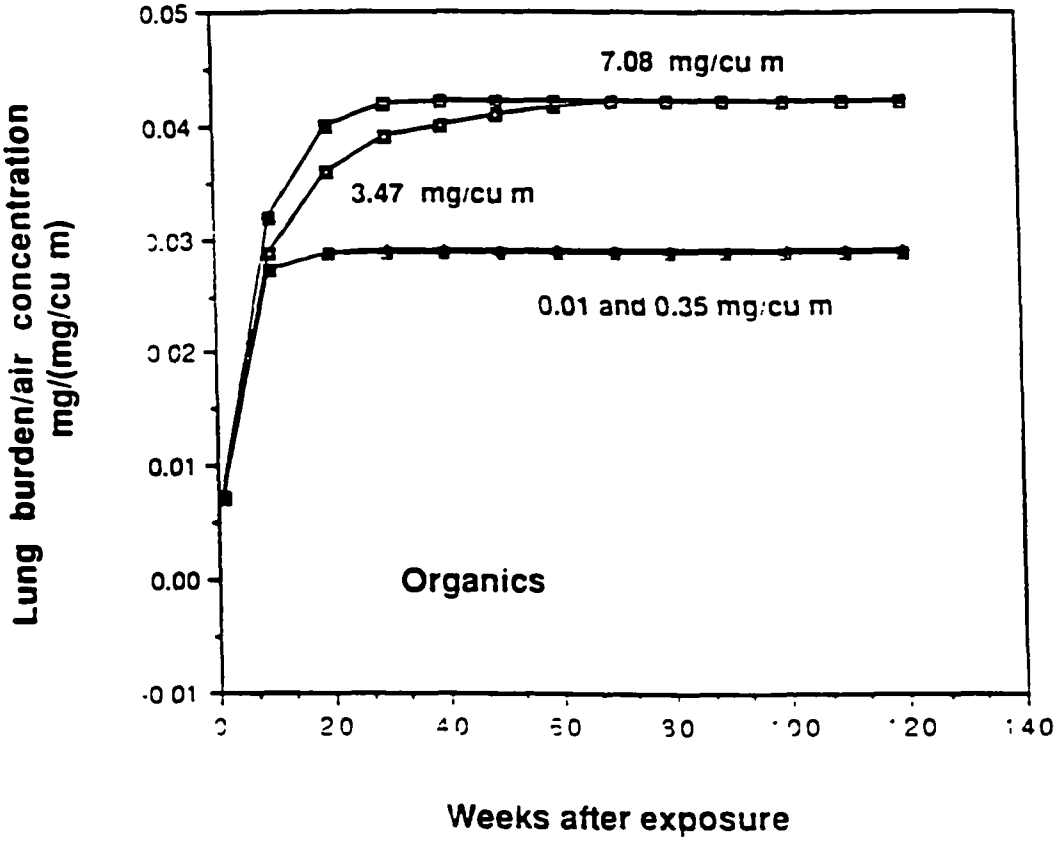

Figure 10-1. Lung burden (organic material, mg) in rats, under exposure scenario (7 h/d, 5 d/week) in Mauderly et al. (1987).

with half of this mass composed of slowly cleared organics and the other half composed of quickly cleared organics.

As shown in Figures 10-1 and 10-2, the steady-state lung burdens are reached much faster for organics than for the carbon core. The steady state in the lung of the retained dose is reached very quickly for organics and will not change during further continuous exposure over 2 yr. However, it is very likely that this continuing exposure after steady state is reached will have a major influence on tumor induction. For particles inhaled at a concentration above a few mg/m^3, retarded clearance will become an increasingly important factor for their accumulated dose over an extended exposure period. Since lung burden of organics reaches steady state very quickly after exposure, the steady state value is used as the 'target' dose in risk calculations. The use of such a dosimetry is practically equivalent to the use of the area under the curve: a dosimetry often used in risk assessment.

For the reasons discussed previously in the introduction, it is reasonable to assume, at present, that only organic material is carcinogenic. Assuming that the same accumulated doses of the absorbed organics per lung epithelial surface area (μg/cm^2) are equivalent between animals and humans, unit risks are calculated, using tumor incidence data from each of the three bioassays (Brightwell et al., 1986; Ishinishi et al., 1986; and the time to event data from Mauderly et al., 1987) and the corresponding doses in terms of organics per lung surface area. The resultant unit risk estimates using the linearized multistage model are given in Table 10-7, ranging from 5.5×10^{-6} to 2.6×10^{-5} with a geometric mean of 1.3×10^{-5}. In these calculations, the relationship between air concentration (μg/m^3) and lung burden (mg) in humans (Figure 10-3) is used to determine human lung burden due to 1 μg/m^3 of diesel engine emissions. For instance, when data from Ishinishi et al. (Table 10-4) are used, the carcinogenic slope (i.e. 95 percent upper confidence of the linear coefficient in the multistage model) expressed in term of equivalent dose (μg/cm^2) is 0.64 per μg/cm^2. Since 1 μg/m^3 of diesel engine emissions in air corresponds to a steady-state lung burden of 5.4 μg per lung, or equivalently 8.6×10^{-6} μg/cm^2 (5.4 μg/627,000 cm^2 lung surface) of organics, the lifetime risk due to 1 μg/m^3 of diesel engine emissions in the ambient air is 5.5×10^{-6} ($0.64\times8.6\times10^{-6}$).

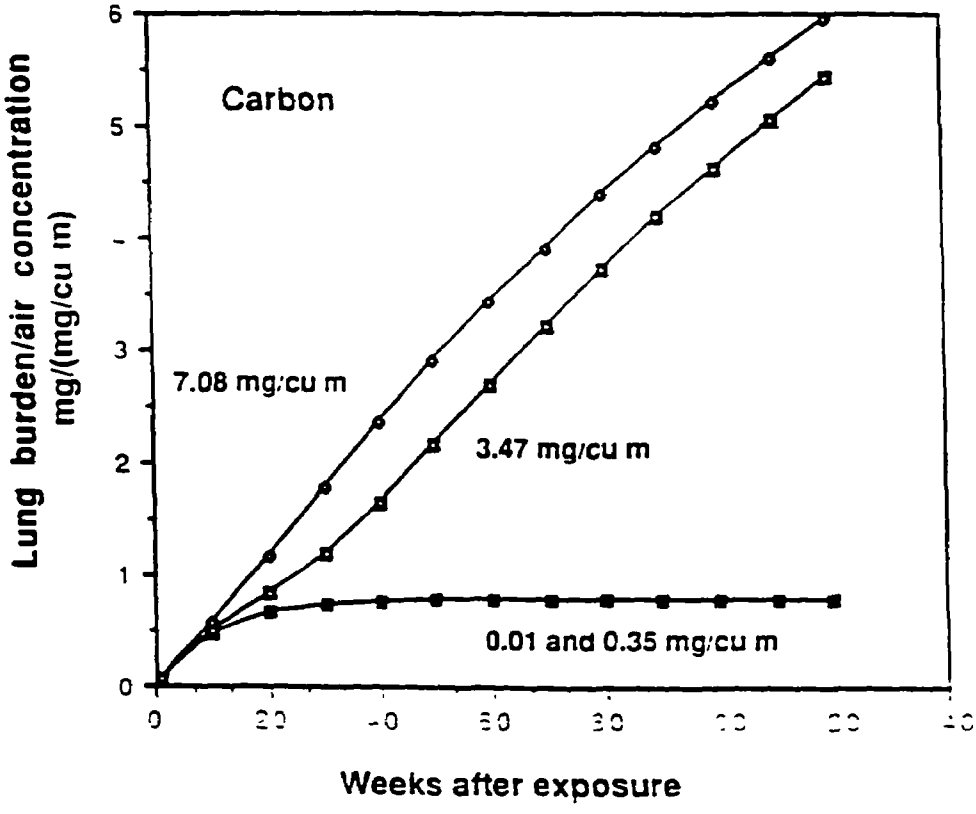

Figure 10-2. Cumulative lung burden (particles, mg) in rats, under exposure scenario (7 h/d, 5 d/week) in Mauderly et al. (1987).

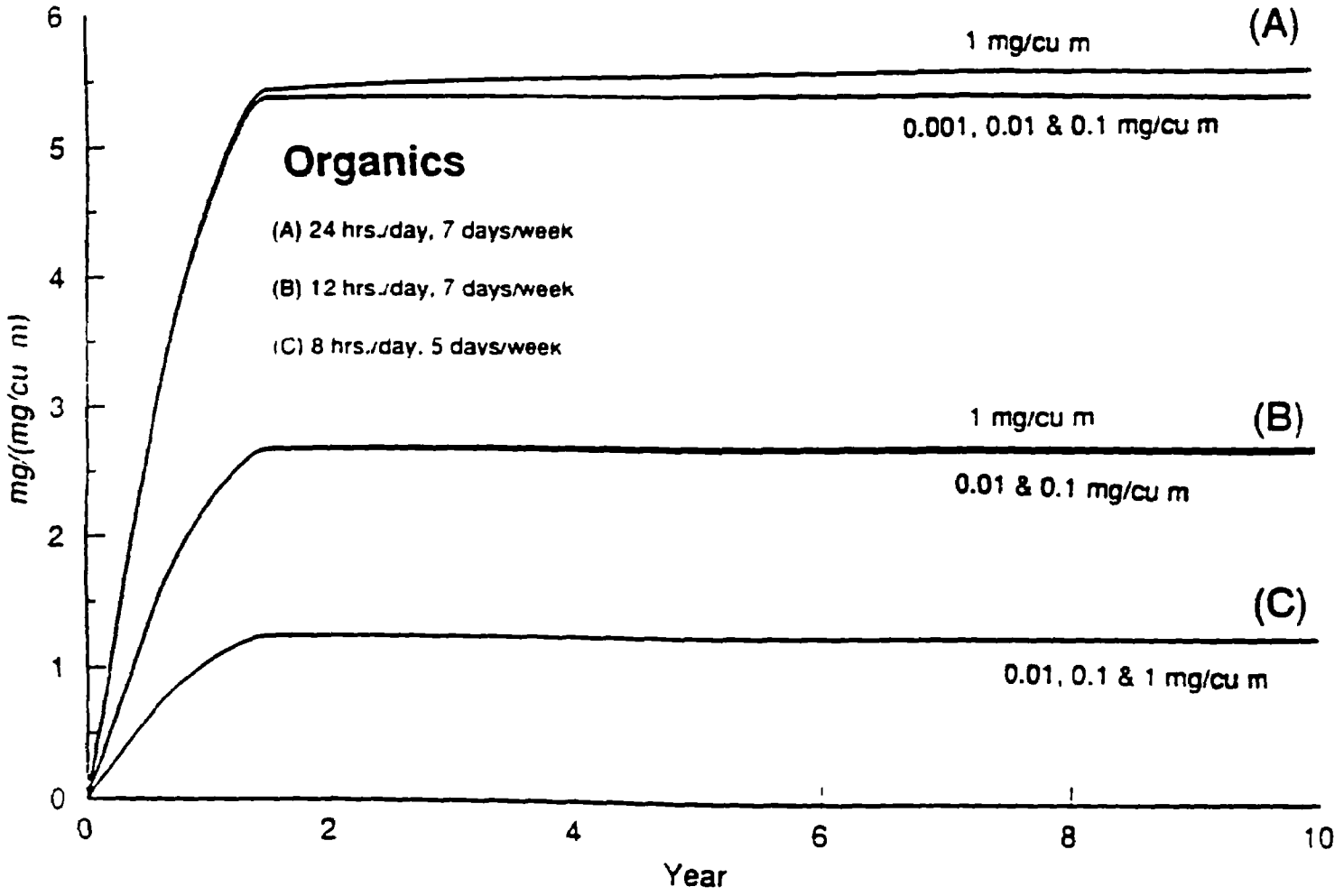

Figure 10-3. Lung burden (organics, mg) in human adults over years after exposure.

TABLE 10-7. UNIT RISK ESTIMATES PER ug/m^3 OF DIESEL EXHAUST

Data base	Equivalent dose assumption		
	Air concentration	Body surface[a]	Organic per lung surface[b]
Mauderly et al., 1987	1.4×10^{-4}	3.7×10^{-4}	2.6×10^{-5}
Ishinish et al., 1986	3.2×10^{-5}	8.5×10^{-5}	5.5×10^{-6}
Brightwell et al., 1986	7.3×10^{-5}	1.9×10^{-4}	1.6×10^{-5}
Geometric mean (rats)	6.9×10^{-5}	1.8×10^{-4}	1.3×10^{-5}
Pooled Fischer rat data[c]	2.3×10^{-5}	6.1×10^{-5}	4.0×10^{-6}

[a] If body weight, instead of body surface, basis is used, the unit risk estimates will be reduced by a factor of 6.

[b] If organic per lung weight, instead of organics per surface, is used as equivalent dose, those risk estimates would be reduced by a factor of 4.

[c] Data from exposure groups in all studies in which the adjusted concentration was equal to or less than 0.73 mg/m^3 were used.

10.3.2.2. Approach 2

Risk estimates are calculated under three dose equivalence assumptions: namely, mg/m^3 (referred to as air concentration equivalence), mg/kg per day (referred to as body weight equivalence), and mg/$W^{2/3}$ per day (referred to as body surface equivalence), where w is body weight in kg. Under a dose equivalence assumption, the same unit of dose (or exposure) is assumed to be equally potent in inducing tumor responses between animals and humans. For instance, when mg/kg per day (i.e., body weight basis) is assumed to be an equivalent dose, it would require (as to be illustrated below) a 2.3 times higher air concentration for humans than for animals to induce the same tumor responses. When the air concentration is 1 $\mu g/m^3$, the body burdens for rats and humans are calculated to be:

1 $\mu g/m^3 \times 10^{-3}$ mg/μg x (0.20 m^3/d)/0.30 kg = 0.00067 mg/kg per day,
where 0.20 m^3/d is the daily air intake volume for a 0.30 kg-rat and,
1 $\mu g/m^3 \times 10^{-3}$ mg/μg$\times$(20 m^3/d)/70 kg = 0.00029 mg/kg per day,
where 20 m^3/d is the daily air intake volume for a 70-kg man.

Thus, under the assumption that mg/kg per day is an equivalent dose, 2.3 (0.00067/0.00029) times more tumors would be induced in rats than in humans when both rats and humans were exposed to diesel emissions at the same air concentration. Note that, in these calculations, the particle overload effect is not considered and tumor response is assumed to be linearly proportional to the body dose.

The unit risk estimates based on the air concentration and the body surface dose equivalence assumptions are listed in Table 10-7. The unit risk estimates on the body weight basis (not presented here) can be derived from those based on the body surface equivalence by dividing by a factor of 6. One of the primary potential sources of error in estimating risk based upon animal exposure to inhaled particulate matter involves slowing of clearance due to lung overloading. The longer residence time can increase bioavailability by allowing for greater desorption of the organic compounds from the particles. The higher concentrations used in these studies were in the range where slower lung clearance would be expected. To partially avoid this source of error and to obtain a better-defined upper-bound estimate, lung

tumor responses were pooled from all groups in all the rat studies in which the adjusted exposure concentration was equal to or less than 0.73 mg/m^3. The pooled estimate of 2.3×10^{-5} (under the air concentration equivalence assumption) and 6.1×10^{-5} (under the body surface equivalence assumption) are based upon data from 773 control and 1208 exposed animals.

The geometric mean of the risk estimates calculated by Approach 1 is comparable to that calculated by Approach 2 when pooled data are used and air concentration dose equivalence is assumed (Table 10-7). In general, Approach 1 produces smaller risk estimates than those calculated by Approach 2, with the largest difference (by about an order of magnitude) occurring when body surface dose equivalence is assumed and non-pooled data are used. The pooled data produce comparable risk estimates to those calculated by Approach 1 because the overload effect is considered in both situations.

In the Approach 1 calculations, the amount of organics per lung surface ($\mu g/cm^2$) is assumed to be dose equivalent. If, instead, the amount of organics per lung weight ($\mu g/g$) is assumed to be equivalent, the unit risk estimate will be reduced by a factor of about 4, the ratio of rat surface area (4090 cm^2)/human surface area (627,000 cm^2) and rat lung weight (1.6 gram)/human lung weight (1065 gram).

10.4. DISCUSSION: UNCERTAINTIES AND RESEARCH NEEDS

An ideal approach to estimate cancer risk due to diesel exhaust exposure would be to consider the delivered dose rate of organic material together with particles as well as the effects of these compounds on initiation, cell proliferation, and progression rates. If such information is available, the models proposed by Moolgavakar and Knudson (1981) and Chen and Farland (1989) can be used to calculate the dose-response relationship. The observation from dose and time to tumor relationship from Mauderly et al. (1987), where tumor incidence increases drastically over time after exposure in the high exposure group but not in the low- and mid-exposure groups, suggests that diesel emissions increase cell proliferation rate with an increase of exposure concentration. (As shown in Table 10-3, the high dose group has a significantly higher proportion of animals that developed tumors than

the other treatment groups. More than 90 percent of tumors in the high dose group were observed after 700 d.) This speculation is based on a theoretical demonstration (Figure 10-4) that, when the mitotic rate for initiated cells is doubled from 0.14/d to 0.28/d and other model parameters remain constant, tumor incidence is drastically increased over time under a two-stage model of carcinogenesis proposed by Chen and Farland (1989), which is a modified version of the one proposed by Moolgavkar and Knudson (1981). The increase of proliferation rate may be due to organic material and particles acting individually or jointly. Since particles probably cannot act as complete carcinogens, organic material is used as the "target" dose in the risk calculations, assuming that particles play a role in the carcinogenic potential of inhaled diesel exhaust only as a vehicle that allows organics to be slowly released (Approach 1). It would be interesting to conduct an initiation-promotion-progression type of study to determine whether or not particles can promote organics-initiated cells and whether the rate of progression is affected by the particle-associated organics.

A prolonged retention of particles occurring during impaired lung clearance does not prolong the retention of the adsorbed PAH because it is cleared from particles and absorbed quickly compared to the long retention half time of particles. Thus, the rate of delivery of the PAH to target cells may be of importance to metabolize these substances. If this delivery rate is temporarily very high, interaction of the PAH with target cells may become less effective. However, more PAH can be metabolized when the same total amount is delivered at a slower rate over a longer time (i.e., when it is slowly released from particles). Studies have shown that B[a]P in association with particles forms more metabolites and induces more lung tumors than does B[a]P alone. These findings are consistent with the concept of rate-dependent absorption.

Several other data gaps must be filled to improve the risk extrapolation from animals to humans. Experimental studies are needed in particular on mechanistic aspects of diesel exhaust carcinogenicity, since extrapolation modeling from one species to another can best be performed when the underlying mode of action is known. The question of tumor sites and their histologic differences (cells of origin) between rats and humans needs to be addressed. The delivery rate of carcinogens to specific cell types needs to be estimated, and

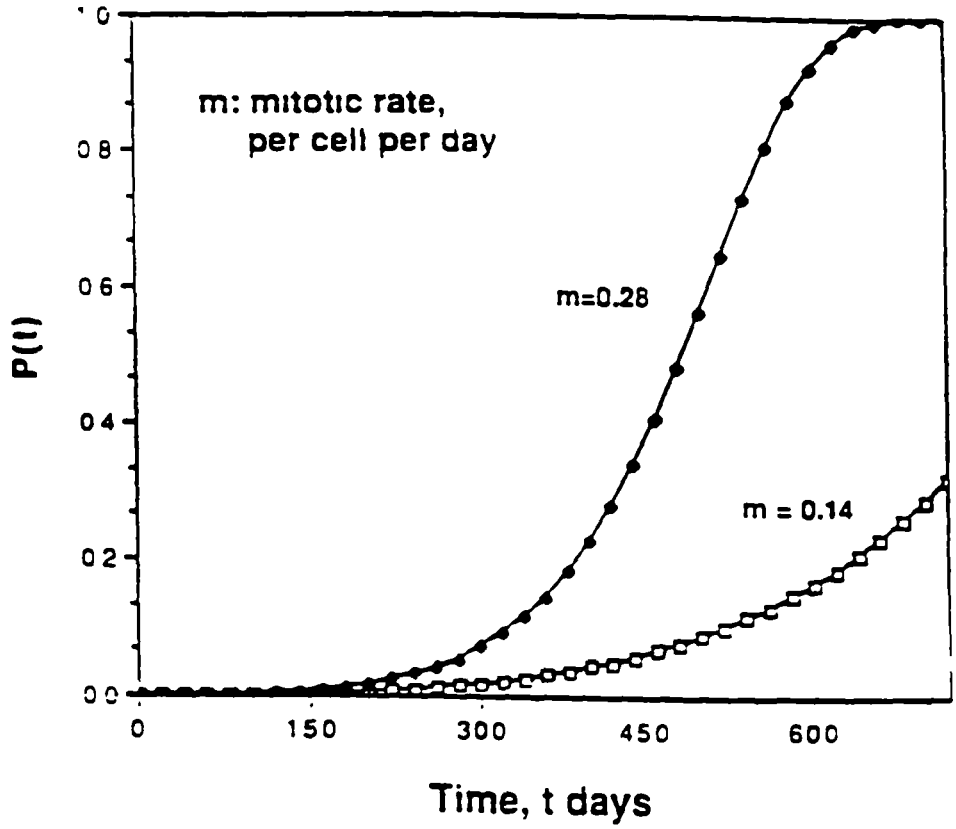

Figure 10-4 Effect of increasing the mitotic rate of initiated cells on the tumor incidence rate over age when other parameters are held constant: A hypothetical calculation based on a two-stage model proposed by Chen and Farland (1989)

normal and initiated cell proliferation rates as well as an effect of exposure on the proliferation rates needs to be investigated. Since we know from the rat studies that alveolar macrophages are activated and that their ability to clear particles is impaired at high inhaled concentrations, other important cellular defense mechanisms might be affected already at low concentrations (e.g., killer cell activity). The generation of mediators from inflammatory cells (e.g., oxygen radicals) could be an important mechanistic aspect in lung tumor induction.

Ongoing studies with inhaled pure carbon black particles will shed light on the role of carbonaceous particles *per se* in lung tumor induction. The potential of diesel exhaust to act as a promoter on both normal and initiated cells deserves attention, and studies on DNA interactions (DNA-adduct formation) and on effects of normal cellular repair mechanisms will be important. Since rats, mice, and hamsters vary in their sensitivity to carcinogens, comparative studies would be useful to elucidate underlying mechanisms. Knowledge of these carcinogenic mechanisms will eventually lead to an improved extrapolation model for the pulmonary carcinogenic effect of inhaled diesel exhaust from rat to humans. Finally, some of the rate constants used in this paper (Appendix C) may need to be confirmed, although the model adequately predicts available data on the lung burden of particles.

10.5 Weight-of-Evidence for Carcinogenicity of Diesel Engine Emissions and Recommended Unit Risk Estimates

Inhalation of whole diesel exhaust resulted in the induction of lung tumors in F344 rats (Mauderly et al., 1987; Ishinishi et al., 1986; Iwai et al., 1986; Brightwell et al., 1986; in Wistar rats Heinrich et al., 1986b; in NMRI mice (Heinrich et al., 1986b); and in Sencar mice (Pepelko and Peirano, 1983). Lung tumors were also induced by implantation of diesel exhaust condensate in Osborne Mendal rats (Grimmer et al., 1987). Skin painting of diesel particle extracts induced dermal tumors in strain "A" mice (Kotin et al., 1955) and in Sencar mice following promotion with TPA (Nesnow et al., 1982). Extensive studies using *Salmonella* have unequivocally demonstrated direct-acting mutagenic activity in both particulate and gaseous fractions of diesel exhaust. Positive results have also been reported

for gene mutations and chromosome effects in mammalian cell systems after exposure to diesel products.

Based upon the induction of lung tumors via inhalation in two strains of rats and two strains of mice; the induction of lung tumors by implantation of diesel exhaust condensate, subcutaneous tumors following injection of exhaust particule extracts, and skin tumors following dermal application of exhaust extracts; and supported by positive mutagenicity results, the evidence for carcinogenicity of diesel exhaust in animals is considered to be sufficient.

Among human populations potentially exposed to diesel engine exhaust, an excess risk of lung cancer was observed in three mortality studies (Garshick et al., 1988; Howe et al., 1983 and Boffetta et al, 1988) and in six case-control studies (Garshick et al., 1987; Benhamon et al., 1988; Hayes et al., 1989; Damber and Larsson, 1987; Hall and Wynder, 1984 and Williams et al., 1977). A dose response was observed in two of the cohort and two of the case-control studies. Although an increased risk of lung cancer was not detected in a few studies (Waller et al., 1981; Rushton et al., 1983; Edling et al., 1987; Wong et al., 1985 and Lerchen et al., 1987), these studies had several methodologic limitations such as small sample sizes, short follow up, lack of adjustment for confounding factors, etc.

Collectively, the epidemiologic studies show a positive assocation between diesel exhaust exposure and cancer. However, because of the uncertainties created by lack of actual diesel exhaust exposure data in these populations, the evidence for carcinogenicity of diesel engine emissions in humans is considered to be limited.

On the basis of limited evidence for carcinogenicity in humans, diesel engine emissions are classified as category B1 according to U.S. EPA cancer assessment guidelines. This classification is supported by sufficient evidence in animals and positive results in mutagenicity studies and is consistent with the presence of known carcinogens such as benzo(a)pyrene on diesel particles. Chemicals classified in category B1 are considered to be probable human carcinogens.

Previous quantitative risk estimates for diesel engine emissions were based upon either the comparative potency method or upon epidemiology data. In the comparative potency method, the effectiveness of diesel exhaust was compared to a related environmental

pollutant, i.e., coke oven emissions, using short term *in vivo* and *in vitro* tests. The relative potency of diesel exhaust under these test conditions was then multiplied by the epidemiology based cancer unit risk estimate for the related pollutant. Confidence in this method is limited by the aggregate uncertainty of the epidemiology based assessments for the related pollutants, by the possibility that the relative potency of the agents compared may be different in short-term tests than in chronically exposed humans, and by the use of extracts rather than whole particles in most of the short-term tests. Confidence in the Harris (1983) epidemiology-based cancer potency estimate is limited because deaths from cancer in exposed workers did not significantly exceed those of the general population, because of limited data regarding exposure levels and changes in exposure with time, because of possible group differences in smoking rates, because of random effects, and because of uncertainties regarding mathematical specification of the model used.

In the derivation of newer potency estimates, several potential uncertainties may still remain, even with the availability of adequately designed and conducted chronic bioassays. One present in all animal data-based assessments is the potential difference in sensitivity of animals vs. humans. Of special concern during diesel exhaust exposure is the inhibition of particle clearance at the high concentrations used in the animal bioassays. This results in greater lung particle burden/exposure concentration ratios than at lower exposure levels. Since the particles could conceivably act as tumor promoters or even as cocarcinogens, unit risk estimates derived from high dose bioassays may overestimate cancer potency at ambient concentrations. Slower particle clearance rates can also result in slightly increased bioavailability of organics even though the half-time for desorption of organics is less than that for normal particle clearance. Finally, the unit risk is based upon particle concentrations and does not account for possible effects of the gaseous fraction of exhaust. Even though carcinogenic effects of the fraction have not been detected, the gases could influence the potency of the particulate matter fraction in some as yet undetected manner.

Two approaches were used in an attempt to circumvent as many of these sources of error as possible. In one approach, inhibition of clearance at high exposure concentrations, with an accompanying excess particulate matter lung burden, was to some degree mitigated by the use of pooled, relatively low level exposure data. The responses at low exposure

concentrations, however, were small and showed considerable variation. The other approach utilized a sophisticated lung dosimetry model that accounted for human-animal differences in regional lung deposition, lung surface area, and effects of particle clearance rates upon the bioavailability of the organic fraction. The precision of the estimate was enhanced further by the use of time-to-tumor data in one of the studies. The two approaches were also combined in that the dosimetry model was used with the pooled, low-level exposure data.

Any of the three unit risk estimates derived from the Mauderly et al. (1987), Ishinishi et al. (1986), and Brightwell et al. (1986) studies, using the dosimetry model (Approach 1), based upon the concentration of organics per unit lung surface area, are considered acceptable. These estimates vary from 5.5×10^{-4} to 2.6×10^{-5}, with a geometric mean of 1.3×10^{-5}. They are considered to be more appropriate than the body surface area method used by EPA in the past, because they provide a more accurate estimate of the delivered dose of organics per surface area of lung rather than lung weight-based estimates and are recommended because they most closely reflects dose at the critical target organ. The estimate based on pooled data from low-level exposures is not recommended because of the low and variable responses in the different studies. Among these estimates, the geometric mean value of 1.3×10^{-5} is considered slightly superior because it represents the largest data base. Among those based on individual studies, the one using Mauderly et al (1987) has the advantages of the longest exposure duration as well as data allowing use of a time-to-tumor model. The estimate of 2.6×10^{-5} based on this data set was also, perhaps fortuitously, exactly the same as the comparative potency estimate reported by Albert et al. (1983).

Whereas estimates based upon the recommended method do not account for overload of the insoluble carbon particle core, there are little data that would allow adjustments to be made. It is even possible that only minimal adjustment would be necessary. Pott and Stober (1983), for example, reported that intratracheal instillation of particulate matter did not enhance the carcinogenic potency of benzo(a)pyrene. Moreover, since mechanical clearance of particles from the alveolar regions is normally much slower in humans than in rats, lung burden/exposure concentration ratios for humans exposed at low doses may not differ that greatly from rats exposed at much higher concentrations.

Confidence in the recommended unit risk estimates are increased by the facts that (1) They are based upon several well designed and conducted chronic animal bioassays (2) time-to-tumor data were available in at least one of the bioassays (3) the major factors responsible for the internal dosimetry of the putative carcinogenic organics are accounted for (4) the risk estimate is based upon whole particulate matter rather than extracts.

Because of remaining uncertainties including possible differing sensitivities between humans and rats, the possible effects of the inorganic fraction of the inhaled particulate matter, and possible effects of the gaseous components, confidence in the recommended unit risk estimate as an upper bound is considered to be medium rather than high.

10.6. SUMMARY

On the bases of limited evidence for carcinogenicity of diesel engine emissions in humans, supported by adequate evidence in animals and positive mutagenicity data, diesel engine emissions are considered to best fit the weight-of-evidence category B1. Agents classified into this category are considered to be probable human carcinogens.

Two approaches and several different equivalence dose assumptions are used to calculate unit risk estimates. Approach one accounted for animal-human differences in particle deposition efficiency, lung surface area, as well as normal and impaired clearance rates upon the lung burden of organics. In approach two, the same exposure level based upon either air concentration, body weight or body surface area was considered to be equally potent for inducing tumors. When particle overload effects of impaired clearance upon lung burden of organics, by using doses at which overload is minimized (pooled data), or by doing both, the following unit risk estimates were calculated.

3.0×10^{-6}, on organics per lung weight basis, by approach 1*

1.3×10^{-5}, on organics per lung surface basis, by approach 1*

1.0×10^{-6}, on organics per lung weight basis, pooled data. by approach 1

4.0×10^{-6}, on organics per lung surface basis, pooled data. by approach 1

1.0×10^{-6}, on body weight basis, pooled data, by approach 2

2.3×10^{-5}, on air concentration basis, pooled data, by approach 2

6.1×10^{-5}, on body surface basis, pooled data, by approach 2

*Geometric mean of unit risks from three studies

Of these unit risk estimates, the first two are the most reasonable because effects of particle overload upon the dose of bioavailable, particle associated-organics are accounted in the calculations. Although pooled data provide comparable risk estimates, it is questionable that pooling data from different studies is appropriate because these studies were not conducted under the same protocol, and secondly because the responses were quite small and variable. Of the first two estimates, the one based on organics per unit lung surface area ($1.3 \times 10^{-5}/\mu g/m^3$ is considered to be the most accurate because it most closely reflects concentration at the critical target organ.

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