EPA/600/8-90/057C
February 1998
SAB Review Draft

Health Assessment Document for Diesel Emissions

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National Center for Environmental Assessment-Washington Office
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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PREFACE

This draft health assessment was prepared by the National Center for Environmental Assessment-Washington Office, which is a risk assessing program in the EPA's Office of Research and Development. This assessment has been prepared for EPA's Office of Mobile Sources which has mandates to consider the health hazards associated with diesel engine use in vehicular transportation. As diesel exhaust emissions also affect air toxics and ambient particulate matter, other EPA air programs also have an interest in this assessment. An earlier draft of this assessment was released for public comment in December 1994, and the Agency's Clean Air Scientific Advisory Committee (CASAC) met in public session in May 1995 to review the draft. This February 1998 draft builds on the 1994-1995 history.

The scientific literature search for this assessment is generally current through December 1997 for the health chapters and is generally current through early 1995 for the background chapters dealing with emission characterization. The health assessment document series is not intended to be encyclopedic, rather the focus is on key studies and thus, the health assessment series is less comprehensive than some other types of assessments prepared by EPA.

This February 1998 version of the assessment will be reviewed a second time by CASAC and then EPA will finalize the document.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Authors

Dr. Ronald Bradow
Atmospheric Research and Exposure Assessment Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Dr. Chao Chen
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Dr. Kenney Crump
Clement International Corporation
Ruston, LA

Dr. Daniel Guth
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Dr. John Johnson
Michigan Technological University
Houghton, MI

Dr. Aparna Koppikar
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Ms. Tammie Lambert
Clement International Corporation
Ruston, LA

Dr. Bruce Lehnert
Pulmonary Biology-Toxicology Section
Los Alamos National Laboratory
Los Alamos, NM

Dr. Samuel Lestz
State College, PA

Dr. Kumar Menon
Pikesville, MD

Dr. Günter Oberdörster
Department of Biophysics
University of Rochester Medical Center
Rochester, NY

Dr. Dennis Opresko
Biomedical and Environmental Information Analysis
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Dr. William Pepelko
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Mr. Chris Rambin
Clement International Corporation
Ruston, LA

Dr. Larry Valcovic
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Mr. Michael Walsh
Arlington, VA

Dr. Ronald K. Wolff
Lilly Research Laboratories
Greenfield, IN

Dr. Robert A. Young
Biomedical and Environmental Information Analysis
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Dr. Barbara Zielinska
Desert Research Institute
Energy and Environmental Engineering Center
Reno, NV

Dr. Chia-Ping Yu
State University of New York at Buffalo
Department of Mechanical and Aerospace Engineering
Buffalo, NY

Project Managers

Mr. William Ewald
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Dr. William Pepelko
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Contributors and Reviewers

The following individuals reviewed the current and/or an earlier draft of this document and participated in a peer review workshop on July 18 and 19, 1990.

Dr. Roy Albert
University of Cincinnati
Cincinnati, OH

Dr. James Bond
Chemical Industries Institute of Toxicology
Research Triangle Park, NC

Dr. Glen Cass
California Institute of Technology
Pasadena, CA

Dr. Eric Garshik
Harvard Medical School
Channing Laboratory
Boston, MA

Dr. Judith Graham
National Exposure Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Dr. Uwe Heinrich
Department of Environmental Hygiene
Fraunhofer Institute
Hanover, Germany

Dr. Joellen Lewtas
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC

Dr. Joe Mauderly
Lovelace Inhalation Research Institute
Albuquerque, NM

Dr. Roger McClellan
Chemical Industries Institute of Toxicology
Research Triangle Park, NC

Dr. Fred Miller
Duke University Medical Center
Durham, NC

Dr. Otto Raabe
University of California
Davis, CA

Mr. Charles Ris
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Dr. Herbert Rosenkranz
Department of Environmental Sciences
School of Medicine
Case Western University
Cleveland, OH

Dr. Irving Salmeen
Ford Motor Company
Scientific Research Laboratory
Dearborn, MI

Dr. Andrew Sivak
Cambridge, MA

Dr. Jeanette Wiltse
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Dr. Thomas Smith
University of Massachusetts Medical Center
Worcester, MA

Dr. Frank Speizer
Channing Laboratory
Boston, MA

Dr. Leslie Stayner
National Institute for Occupational Safety and Health - Taft Laboratories
Cincinnati, OH

Dr. Werner Stöber
Chemical Industries Institute of Toxicology
Research Triangle Park, NC

Dr. Jaroslav Vostal
EHAC International
Bloomfield Hills, MI

Dr. James Walker
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

ACKNOWLEDGMENTS

Word Processing Support

Ms. Debra Butler
The CDM Group, Inc.
Chevy Chase, MD

Ms. Glenda Johnson
Biomedical and Environmental Information Analysis
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Document Production

Ms. Theresa Konoza
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Ms. Kay Marshall
Mr. Eric Sorensen
The CDM Group, Inc.
Chevy Chase, MD

Ms. Marianne Barrier
Mr. John Barton
Ms. Sheila Lassiter
Ms. Wendy Lloyd
Ms. Edie Smith
ManTech Environmental Technology, Inc.
Research Triangle Park, NC

References

Mr. Douglas Fennell
Environmental Criteria and Assessment Office
U.S. Environmental Protection Agency
Research Triangle Park, NC

Ms. Catherine Carter
Ms. Blythe Hatcher
Information Organizers, Inc.
Research Triangle Park, NC

Reprographics

Mr. Richard Wilson
Environmental Criteria and Assessment Office
U.S. Environmental Protection Agency
Research Triangle Park, NC

1. INTRODUCTION

Diesel engines are an important part of transportation and industry throughout the industrialized world, and their use may expand because of increased pressures for fuel efficiency. For example, in addition to their widespread use in heavy-duty trucks and in many nonroad applications (such as construction and agriculture), there is increased use of diesel engines in light/medium duty trucks. Also, new technologies, such as hybrid vehicles powered by advanced diesel engines, and the potential for increased use of light-duty diesel engines in the sport utility market could fuel this expansion. In both the United States and Europe (where diesel passenger vehicles are much more common than in the United States), decisions are being made about how to regulate diesel engine and diesel fuel emissions; these decisions need to be based on sound scientific foundations.

EPA's Office of Mobile Sources (OMS) has traditionally regulated diesel engine emissions through Clean Air Act (CAA) provisions, based on diesel engines' contribution to nonattainment of the PM-10 and ozone National Ambient Air Quality Standards (NAAQS). In particular, diesel engines are a significant source of particulate and nitrogen oxide (NO_x) emissions. Since the passage of the first CAA in 1977, great strides have been made in controlling particulate matter and NO_x emissions (including NO and NO_2, both of which contribute to ozone formation) from highway and nonroad diesel engines. Heavy-duty urban bus diesel engine particulate certification standards decreased by approximately 90% between 1988 and 1996, and heavy-duty highway diesel engine particulate certification standards decreased by approximately 80% in the same period. NO_x emissions standards have also been reduced, resulting in significant control of these emissions over the years. Manufacturers have reduced particulate NO_x emissions by a variety of control technologies, such as altered combustion chamber design, better fuel injection technology such as high-pressure injection, and oxidation-type catalysts in some cases. EPA has also implemented initial regulations controlling emissions (NO_x) from nonroad diesel engines. In addition, EPA has regulated diesel fuel quality by limiting the sulfur content of diesel fuel, thereby reducing particulate emissions.

New authority granted to OMS under the CAA Amendments of 1990 allows for further regulation of nonroad diesel engines. Particulate and NO_x emission standards have been or will be established for diesel engines used to power nonroad equipment such as construction equipment, marine vessels, and locomotives.

As mentioned previously, EPA has taken action to control diesel engine particulate matter emissions in order to facilitate attainment with the PM-10 NAAQS as a direct result of the adoption of a new NAAQS for particulate matter less than 2.5 μm in diameter. On the basis of

current health research, it is this fraction of the PM that is implicated in both the cancer and noncancer effects observed from diesel particulate emissions exposure. PM 2.5 data indicate that diesel engines contribute significantly to the national particulate emission inventories. Further regulation of diesel engine particulate emissions will require sound scientific evidence from the NAAQS effort as well as continuing research related to particulate effects on public health.

EPA has never prepared a comprehensive health assessment document focused on the possible health hazards from direct exposure to diesel exhaust emissions. Piecemeal health assessment work was done at various times in the 1980s, as was EPA-sponsored health research, with most of the attention focused on the possible carcinogenicity of DE exposure. The purpose of this assessment is to capture the key data relating to toxicity and to assess that body of evidence in order to produce a risk characterization describing the human health hazards of DE exposure. Companion comprehensive characterizations of human exposure, diesel engine emission factors, postemission DE pollutant transformation, and transport in the ambient air are not included in the assessment, though some overview information is provided so that the health assessment findings can be placed in a context with related issues.

In Chapters 2 through 11 of this assessment, key human and animal toxicity topics, as well as overview topics, are reviewed and conclusions drawn as appropriate. Chapter 12 integrates the various findings about the potential for health hazards and risk and additionally provides a risk characterization.

The health assessment findings from this assessment will be advisory to EPA's Office of Mobile Sources by providing health impact information that can be used to characterize residual health hazards and risk.

2. DIESEL EMISSIONS, TRANSPORT, AND TRANSFORMATION

2.1. INTRODUCTION

This chapter reviews a number of background topics that are valuable for perspective and context as one considers the health data (in later chapters) for diesel exhaust exposure. The contents of this chapter are not intended to necessarily reflect the most current literature, and thus should not be considered comprehensive and authoritative in that respect.

The diesel engine was patented in 1892 by Rudolf Diesel, who conceived it as a prime mover that would provide much improved fuel efficiency compared with spark-ignition engines. To the present day, the diesel engine's high efficiency remains its strongest selling point. In the United States, the diesel engine is used mainly in trucks, buses, agricultural and other off-highway equipment, locomotives, ships, and many stationary applications.

The chief advantages of the diesel engine over the gasoline engine are its fuel economy and durability. Diesel engines, however, emit more oxides of nitrogen (NO_x) and carbonaceous particulate matter than do gasoline engines. Over the past decade, modifications of diesel engine components have substantially reduced gaseous and particle emissions (Hammerle et al., 1994).

The diesel engine compresses air to high pressure and temperature. Fuel, when injected into this compressed air, autoignites, releasing its chemical energy, and the resulting combustion gases expand, doing work on the piston, before being exhausted to the atmosphere. Power output is controlled by the amount of injected fuel rather than by throttling the air intake. Compared to its spark-ignited (SI) counterpart, the diesel engine's superior efficiency derives from a higher compression ratio and no part-load throttling. Because of its poorer air utilization, a diesel engine requires a larger piston displacement for the same power output as a comparable SI engine. To ensure structural integrity for prolonged reliable operation at the higher peak pressures brought about by a higher compression ratio and autoignition, the structure of a diesel engine generally is more massive than its SI counterpart.

Diesel engines may be broadly identified as being either two- or four-stroke cycle, injected directly or indirectly, and naturally aspirated or supercharged. They also are classified according to service requirements such as light-duty (LD) or heavy-duty (HD) automotive, small or large industrial, and rail or marine engines.

All diesel engines use hydraulic fuel injection in one form or another. The fuel system must meet four main objectives if a diesel engine is to function properly over its entire operating range: (1) meter the correct quantity of fuel, (2) distribute the metered fuel to the correct cylinder, (3) inject the metered fuel at the correct time, and (4) inject the fuel so that it is atomized and mixes well with the in-cylinder air. The first two objectives are functions of a well-designed injection pump, and the last two are mostly functions of the injection nozzle. As a

part of the effort to obtain lower exhaust emissions without diminishing fuel efficiency, fuel injection systems are moving toward the use of electronics for more flexible control than is available with purely mechanical systems.

Both the fuel and the lubricants that are used to service diesel engines are highly finished petroleum-based products combined with chemical additives. Diesel fuel oil is a mixture of many different hydrocarbon molecules from about C_7 to about C_{35}, with a boiling range from roughly 350°F to 650°F. Many of the fuel oil properties, such as its specific energy content, ignition quality, and specific gravity, are related to its hydrocarbon composition. Therefore, fuel and lubricant composition affects many aspects of engine performance, including economy and exhaust emissions. For example, a decrease of fuel aromatic content, sulfur, and volatility usually leads to a reduction of regulated emissions (Ullman, 1989). The four stages of the combustion process in the diesel engine are:

1. Ignition delay period: the elapsed time from the start of injection until the start of combustion. This is the time required to atomize fuel, evaporate droplets, and mix vapor with air and for the necessary preflame reactions to occur. The ignition delay period is really two inseparable overlapping delay periods, physical delay and chemical delay.

2. Uncontrolled burning period: during this stage the fuel that has passed entirely through the first stage autoignites and burns in a premixed fashion and then diffusion takes over control of the burning (Lyn, 1963; Kahn, 1970). During this stage a high rate of pressure rise and noise associated with diesel knock occur.

3. Controlled burning period: during this stage the fuel burns as it is injected in what is essentially a diffusion-controlled process. The burning rate and the rate at which energy is released in this stage are lower than during the second stage.

4. Afterburning period: the elapsed time from the end of fuel injection until the end of combustion. This stage is characterized by the diffusion mode giving way to the premixed mode of combustion.

Diesel emissions are derived from the complete and incomplete combustion of fuel and lubricating oil; they are a mixture of gases and low molecular weight (MW) carbon particles. High MW organic compounds are adsorbed on the particles. Table 2-1 lists the major diesel combustion emissions and their atmospheric reaction products.

Combustion of fuel in the diesel engine results in the formation of a complex mixture of gaseous and particulate exhaust. Because of concerns over possible health effects associated with diesel particulate emissions, measurements have been made to characterize chemically in detail the exhausts from light-duty diesel (LDD) and, to a lesser extent, heavy-duty diesel (HDD)

Table 2-1. Major components of diesel engine emissions and their known atmospheric transformation products

Emission component	Atmospheric reaction products
A. Vapor-phase emissions[a]	
Carbon dioxide	—
Carbon monoxide	—
Oxides of nitrogen	Nitric acid, ozone
Sulfur dioxide	Sulfuric acid
Hydrocarbons Alkanes ($\leq C_{18}$) Alkenes ($\leq C_4$) (e.g., 1,3-butadiene)	 Aldehydes, alkyl nitrates, ketones Aldehydes, ketones
Aldehydes Formaldehyde Higher aldehydes (e.g., acrolein)	 Carbon monoxide, hydroperoxyl radicals Peroxyacyl nitrates
Monocyclic aromatic compounds (e.g., benzene, toluene)	Hydroxylated and hydroxylated-nitro derivatives[b]
PAHs ($\leq$4 rings)[c] (e.g., phenanthrene, fluoranthene)	Nitro-PAHs ($\leq$4 rings)[d]
Nitro-PAHs (2 and 3 rings) (e.g., nitronaphthalenes)	Quinones and hydroxylated-nitro derivatives
B. Particle-phase emissions	
Elemental carbon	—
Inorganic sulfate	—
Hydrocarbons (C_{14}-C_{35})	Little information; possibly aldehydes, ketones, and alkyl nitrates
PAHs ($\geq$4 rings) (e.g., pyrene, benzo[a]pyrene)	Nitro-PAHs ($\geq$4 rings)[d] Nitro-PAH lactones
Nitro-PAHs ($\geq$ 3 rings) (e.g., nitropyrenes)	Hydroxylated-nitro derivatives

[a]Unless otherwise stated, the impact results from both the emissions components and the atmospheric reaction products.
[b]Some reaction products expected to partition into the particle phase.
[c]PAHs containing four rings are usually present in both the vapor and particle phases.
[d]Nitro-PAHs with more than two rings will partition into the particle phase.

Source: HEI, 1995.

engines. Most of these measurements are of primary pollutants, that is, gases and particulate matter emitted directly into the air from their sources.

The primary pollutants are subject to dispersion and transport and, at the same time, to chemical and physical transformations into secondary pollutants; the time scales of these atmospheric transformations and physical loss processes vary widely. Atmospheric lifetimes range from <1 min for some highly reactive organic compounds to months for other much more inert constituents of direct emissions. Thus, to assess the environmental effects of diesel emissions, it is necessary to determine the chemical and physical changes that primary diesel emissions undergo during their transport through the atmosphere.

Combustion of fuel in the diesel engine results in the formation of a complex mixture of gaseous and particulate exhaust. Because of concerns over possible health effects associated with diesel particulate emissions, measurements have been made to characterize chemically in detail the exhausts from light-duty diesel (LDD) and, to a lesser extent, heavy-duty diesel (HDD) engines. Most of these measurements are of primary pollutants, that is, gases and particulate matter emitted directly into the air from their sources.

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2.2. OVERVIEW OF DIESEL POLLUTANTS AND POLLUTANT FORMATION

2.2.1. Gas-Phase Pollutant Emissions

2.2.1.1. *Oxides-of-Nitrogen Formation*

Because the diesel combustion process is very complex and involves burning of fuel droplets, it has proven difficult to predict pollutant concentrations or emission rates quantitatively. In SI gasoline engines, NO emission can be quantitatively explained by adiabatic compression of the initially burned mixture (nearest the spark plug) by the combustion pressure developed in the later stages of combustion. Thus, the originally burned gases are raised to a much higher temperature than that achieved in the flame by the subsequent compression. Shahed (1985) has reviewed the work on this subject for diesel engines. He reports that, qualitatively, NO formation in diesel engines cannot be explained by this phenomenon. The time-temperature history of the burning droplets seems to determine the extent of NO formation. Yu and Shahed (1981) have defined relevant engine operating parameters that control NO emissions for heavy-

duty diesel engines. For instance, retarding injection or recirculating exhaust gases reduces NO formation and emission at the expense of increasing soot formation and hydrocarbons, all other factors being equivalent. Wu and Peterson (1986) studied NO formation kinetics in an IDI passenger-car diesel engine over a wide range of operating conditions. These authors found that a variable-temperature model accounting for the average gas temperature at the time of droplet burning explained the observed NO considerably better than a constant-temperature (peak-cycle-temperature) model. Global NO formation rates in their study suggested that the NO must be formed in the vicinity of the droplet flame zone.

Lipkea et al. (1987) and Lipkea and DeJoode (1987) have constructed a successful engine model that adequately explains NO formation. Process parameters that control NO formation include fuel jet momentum flux, in-cylinder air density at the start of injection, swirl cross-flow momentum flux, and in-cylinder temperature. Air system design characteristics that change air density and temperature can result in constant work but decreasing NO_x. On the other hand, decreasing cylinder temperatures also result in increased hydrocarbon emissions (Uyehara, 1987; Gill, 1988). The net result is a tradeoff between NO_x and particulate material at high temperatures and between NO_x and hydrocarbons at low operating temperatures.

2.2.1.2. *Hydrocarbons and Carbon Monoxide Formation*

Small quantities of gaseous unburned hydrocarbons (HCs) and carbon monoxide (CO) are emitted from diesel engines, but less than those emitted by comparable SI engines. Myers and Uyehara (1947) have explained the observed CO on the basis of locally rich combustion. During the ignition delay period, especially small amounts of fuel vaporize from the initial droplets. The gas-phase reactions of this material are responsible ultimately for its ignition and, thus, the ignition of the droplets. However, this premixed patch is likely to be locally quite rich, even though the overall fuel-air mixture has considerable excess air. Therefore, CO is formed in concentrations of 2,000 ppm or even slightly more in diesel exhaust. By comparison, typical gasoline engines might have exhaust CO concentrations of 10,000 to 20,000 ppm.

These locally rich combustion processes are also responsible for a small release of low molecular weight hydrocarbons, principally methane, ethylene, and acetylene, in diesel exhaust. About 10% of these materials are C_1 to C_4 combustion-derived compounds. The bulk of the emission in the gas phase is diesel fuel in the C_{10} to C_{25} molecular weight range; these materials account for 70% to 80% of the HCs emitted. The balance of the material, including particle-bound HC, is in the same molecular weight range as lubricating oil. Similar findings have been reported with HD diesel engines, both of the two-stroke and four-stroke cycle types. Therefore, both fuel and lubricant can supply organic matter to diesel engine exhaust HCs in the gaseous and particulate states.

Hampton et al. (1983) reported a gas chromatography-mass spectrometry (GC-MS) study of heavy HCs in a Pennsylvania Turnpike roadway tunnel that has varying amounts of diesel and gasoline passenger-car traffic. There were characteristic differences in gaseous HC content of the tunnel gases between the diesel and gasoline vehicles. Typically, diesel traffic was characterized by substantial quantities of aliphatic hydrocarbons with lesser amounts of long-chain substituted monoaromatics. Gasoline traffic was dominated by methyl- and ethyl-benzene emissions, based on the porous polymer trapped samples used in this study. These results are in general agreement with the Black and High (1979) conclusion that diesel HC emissions are primarily fuel-derived.

2.2.2. Particle Formation and Emission

The chemical mechanism that accounts for carbon formation in diesel combustion is not completely established; the major weight of scientific opinion seems to support some role for intermediate formation of polycyclic aromatic hydrocarbons in the process. Diffusion flames, whether rich or lean, usually form some carbon. However, carbon is consumed on the lean side by reaction with hydroxyl (OH) radicals (Fenimore and Jones, 1967), and only when the OH radical population is reduced by other reactions (with fuel hydrocarbons, for example) is carbon found to be a major combustion product. Thus, carbon is normally a stable combustion product only of rich flames.

Beck and Uyehara (1988) have pointed out that there is a very good linear correlation between CO emission rate and carbon emission rate from HD engines. Once formed, both substances are difficult to remove, requiring highly energetic OH radicals for reaction. These authors argue that carbon formation normally takes place over a rather narrow temperature range and that the maximum rate of carbon formation is found at temperatures around 2,250 K. At temperatures of 2,400 K and above, carbon is burned out, and at temperatures of 1,900 K and below, it is never formed. However, if attempts are made to raise temperature and thus burn out the carbon, high NO results. If flame zone temperatures are lowered by adding water, alcohol, or exhaust gas, HC emissions are increased (Ball, 1987; Kadota and Henein, 1981). Beck and Uyehara have described a qualitative model of droplet combustion in which the burning rate of fuel droplets is controlled by boiling rate. It is shown that an ideal condition involves high-pressure, high-velocity injection with a minimum ignition-delay period. Carbon can be minimized by reducing rich gaseous combustion and NO can be controlled by delayed-injection timing. Under these conditions, the fuel cetane number might become a critical parameter controlling the ignition delay and the region of uncontrolled combustion. In practice, Ullman (1989) has shown that reducing aromatic content and, hence, decreasing the ignition delay of diesel fuel is beneficial in the control of particulate material and of NO_x emissions in three HD engines built to meet the 1988 California or 1991 Federal emissions standards. Tosaka et al.

(1989) have studied the effect of fuel aromatics in promoting diesel carbon formation. These authors have found an aliphatic radical-benzene condensation process that apparently accounts for the additional amounts of carbon that result in the diesel combustion of aromatic fuels.

2.2.3. Gas-to-Particle Conversion

2.2.3.1. *Condensation of Organic Matter*

Generally, the formation of carbon particles is thought to involve growth of particles by polymerization of gaseous intermediates at the surface of small particles (Kadota and Henien, 1981; Plee et al., 1981). Thus, the growth and agglomeration of carbon particles are probably gas-to-particle processes. Ross et al. (1982) studied the properties of diesel particles obtained from an engine operated on high-purity dodecane. In this case, the fuel was too volatile to have an impact on particle composition. These authors found that the carbon particles contained an HC film, which must have been condensed from the gas phase. The carbon had rather low specific surface area, about 0.5 m^2/g, which could be materially increased by high-temperature treatment.

Heats of sorption of a variety of HCs were determined by a GC technique. It was found that the absorptivity of gaseous organic compounds on these particles was controlled by Henry's law of absorption in the organic surface film. The presorbed organic layer was several layers thick, and this material essentially acted as an organic droplet, dissolving materials from the gas phase. Thus, the heat of sorption was adequately explained by the heat of vaporization of the organics (Ross et al., 1982).

2.2.3.2. *Oxidation of Sulfur Oxides*

Studies of diesel particle composition have produced some information about the fate of fuel sulfur. In the earliest studies, sulfate was found to be a significant component of diesel particles (Hare et al., 1976). Generally, the sulfate found in particles accounted for only about 2% of the fuel sulfur charged, the balance being emitted as sulfur dioxide (SO_2).

Sulfate emission rates have been measured using both engine and chassis dynamometer test procedures. Hare et al. (1976) measured composite particle emission rates on the older Federal compliance test for HD engines and found that with overall emission rates from 0.3 to 1.0 g/kW h (0.4 to 1.3 g/bhp h) of particle mass, sulfate emissions were relatively constant at about 0.02 g/kW h (0.03 g/bhp h). Dietzmann et al. (1980) measured sulfate emission rates from a number of HD vehicles driven over simulated urban driving schedules and found emission rates from about 0.03 to 0.05 g/km for overall particle emission rates from 0.5 to 1.6 g/km. Thus, with previous engines, sulfate was a significant but small (2% to 3%) component of diesel particle mass. With newer engine designs and particle emission rates characteristically below 0.25 g/bhp

h, this emission rate amounts to 10% to 15% of the emitted particle mass, a very important portion of the allowable limit. Currently, no means of reducing this sulfate formation is available other than reducing the sulfur concentration of diesel fuel.

Earlier in this document, the evidence regarding the oxidation of nitrogen to nitric oxide was documented. Dietzmann et al. (1980) have reported analysis of HD diesel vehicle exhaust for NO_2 during simulated urban driving. These authors have reported NO_2 concentrations from about 1 to 5 ppm, accounting for 2% to 5% of the NO emitted. Harris et al. (1987) have measured NO_2 and HNO_3 in air-diluted diesel exhaust. In the exhaust from HD engines, the NO_2 concentration was from 1 to 30 ppm, whereas the HNO_3 ranged from 0.08 to 0.8 ppm. Generally, HNO_3 accounted for a few percentage points of the NO_2, which in turn accounted for a few percentage points of the NO.

2.2.4. Nitroarene Formation

The soluble extract from diesel-generated particulate material was shown to cause mutations when subjected to a bacterial assay (Huisingh et al., 1978). It was not long before evidence began to point toward the class of compounds consisting of nitrated polycyclic aromatic hydrocarbons (PAHs) that have come to be called nitroarenes (Pederson and Siak, 1981; Newton et al., 1982). Hare and Bradow (1979) reported a summary of the EPA findings to that date on the formation of semipolar mutagens in both HD and LD diesel engines. Dietzmann et al. (1980, 1981) reported emissions of mutagenic material from a variety of HD trucks operated over transient driving cycles, and Gibbs et al. (1980) reported the emission of bacterial mutagens from a large number of in-use diesel passenger cars.

Very-high-resolution organic analytical procedures have been applied to diesel exhaust samples (Liberti et al., 1984; Schuetzle and Frazier, 1986; Schuetzle and Perez, 1983). Generally, a variety of nitrated polycyclic aromatic compounds has been found, which accounts for a substantial portion of the mutagenicity found. However, not all the bacterial mutagenicity has been identified in this way, and the identity of the remainder of the mutagenic compounds remains unknown. The nitrated aromatics thus far identified in diesel exhaust were the subject of review in the International Agency for Research on Cancer (1989) monograph on diesel exhaust (Table 2-2).

2.3. PRIMARY DIESEL EMISSIONS

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, as well as in chemical analysis methodology. It is likely that emissions from currently manufactured diesel vehicles are not the same as those measured and

Table 2-2. Some nitroarenes identified in vehicle exhaust[a]

1,3-Dihydroxynitropyrene-1
2,5-Dinitrofluorene
2,7-Dinitrofluorene
2,7-Dinitro-9-fluorenone
1,3-Dinitropyrene
1,6-Dinitropyrene
1,8-Dinitropyrene
9-Methylcarbazole
1-Nitro-3-acetoxypyrene
9-Nitroanthracene
2-Nitroanthracene or -phenanthrene
x-Nitroanthracene or -phenanthrene (two isomers)[b]
6-Nitrobenzo[a]pyrene
x-Nitrobenzoquinoline[b]
2-Nitrobiphenyl
3-Nitrobiphenyl
4-Nitrobiphenyl
1-Nitrochrysene
x-Nitrodibenzothiophene (two isomers)[b]
x-Nitro-y,z-dimethylanthracene or -phenanthrene (five isomers)[b]
1-Nitrofluoranthene
3-Nitrofluoranthene
7-Nitrofluoranthene
8-Nitrofluoranthene
2-Nitrofluorene
3-Nitro-9-fluorenone
10-Nitro-1-methylanthracene or -phenanthrene
10-Nitro-9-methylanthracene or -phenanthrene
x-Nitro-y-methylanthracene or -phenanthrene[b]
1-Nitro-2-methylnaphthalene
3-Nitro-1-methylpyrene
6-Nitro-1-methylpyrene
8-Nitro-1-methylpyrene
1-Nitronaphthalene
2-Nitronaphthalene
2-Nitrophenanthrene
1-Nitropyrene
5-Nitroquinoline
8-Nitroquinoline
x-Nitroterphenyl[b]
x-Nitro-y,z,z′-trimethylanthracene or -phenanthrene (six isomers)[b]
x-Nitrotrimethylnaphthalene (three isomers)[b]

[a]From International Agency for Research on Cancer, 1989.
[b]x, y, z, and z′ imply position is unknown.

reported earlier. When possible, the latest data were used; however, the data presented in this chapter should not be considered fully representative of emissions from the wide range of diesel engines currently used or those that may have occurred in the past.

2.3.1. Gaseous Emissions

Diesel passenger-car and light-truck emissions of CO and total gaseous hydrocarbons (THC) are considerably lower than those of gasoline vehicles. For HDD vehicles, the CO and THC emission rates are somewhat lower than, but comparable with, those of HD gasoline vehicles, but NO_x emissions are many times those of average traffic. In addition to these regulated pollutants, diesel exhausts also contain some sulfur dioxide because of the presence of sulfur in the diesel fuel. Following combustion, approximately 98% of the sulfur is emitted as SO_2 and 2% as particulate sulfate (Pierson et al., 1978, 1979; Truex et al., 1980). Most of the sulfate is in the form of sulfuric acid (H_2SO_4) (Truex et al., 1980).

The atmospheric concentration of nitric acid (HNO_3) from LDD exhausts has been reported to be negligible in comparison with that from other anthropogenic sources (Okamoto et al., 1983; Harris et al., 1987). A range of concentrations from ≈100 ppbv (≈250 μg/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, 1983a). The highest value for NH_3 (25 mg/km) was reported for HDDs; ≈4 mg/km was reported for LDD, and ≈10 mg/km and ≈5 mg/km for gasoline-powered vehicles, with and without catalyst, respectively (Pierson and Brachaczek, 1983a). The emission rates of total aliphatic amines were reported to be below the detection limit of 0.04 to 0.3 mg/km for gasoline-powered vehicles and 0.08 to 0.7 mg/km for heavy-duty trucks. It was concluded that motor vehicles are an insignificant source of atmospheric NH_3 and that amines emitted from motor vehicles cannot give rise to carcinogenic nitrosoamines in the amount said to exist in ambient samples (Pierson and Brachaczek, 1983a).

In addition, low concentrations of phenols have been reported in HDD and LDD emissions (Hare and Baines, 1979; Hare and Bradow, 1979). Aliphatic carboxylic acids (mainly formic, acetic, propionic, and benzoic acids) were also reported in vehicle exhausts (Kawamura et al., 1985; Rogge et al., 1993).

Table 2-3 compares the emission rates of some representative alkanes, alkenes, aromatic hydrocarbons, and aldehydes from HDD and LDD engines and gasoline engines with and without a catalytic converter. Data on catalyst-equipped gasoline vehicles were obtained with a chassis dynamometer and are averaged from 46 in-use passenger cars, 1975 to 1982 models, selected to be representative of vehicles actually driven by the U.S. public (Sigsby et al., 1987). Table 2-3

Table 2-3. Emission rates of volatile organic compounds (VOC) from diesel and gasoline engines

	VOC (g/mi [g/km])			
	Diesel		Gasoline	
VOC	HDD	LDD[a]	Catalyst[b]	Noncatalyst[c]
THC	3.65 (2.28)[d]	0.23 (0.14)[e]	1.8 (1.2)	5.4 (3.4)
Methane	NA	0.01 (0.008)	0.26 (0.16)	0.27 (0.17)
Ethylene	NA	0.04 (0.03)	0.14 (0.09)	0.3 (0.2)
Acetylene	NA	NA	0.04 (0.02)	0.26 (0.16)
Propylene	NA	0.01 (0.008)	0.04 (0.03)	0.15 (0.09)
n-Pentane	NA	NA	0.03 (0.02)	0.09 (0.06)
iso-Pentane	NA	NA	0.07 (0.04)	0.27 (0.17)
n-Decane	0.01 (0.007)[f]	NA	0.003 (0.0016)[g]	
n-Dodecane	0.027 (0.017)[f]	NA	0.003 (0.002)[h]	
Benzene	0.024 (0.015)[d]	0.02 (0.015)[i]	0.06 (0.04)	0.31 (0.19)
Toluene	0.01 (0.007)[f]	0.006 (0.004)[e]	0.1 (0.07)	0.7 (0.45)
Xylenes	0.006 (0.004)[d]	0.002 (0.001)[e]	0.08 (0.05)	0.96 (0.6)
Ethyl benzene	0.005 (0.003)[f]	0.001 (0.0006)[e]	0.02 (0.01)	0.21 (0.13)
Naphthalene	0.01 (0.007)[f]	0.003 (0.002)[e]	NA	NA
Formaldehyde	NA	0.02 (0.01)	0.025 (0.015)	0.06 (0.04)[i]
Acetaldehyde	NA	0.007 (0.004)[e]	0.01 (0.007)	NA
Acrolein	0.053 (0.033)[d]	0.01 (0.006)	0.002 (0.001)	NA
Benzaldehyde	NA	NA	0.003 (0.002)	NA
Total aldehyde	NA	0.03(0.02)[e]	0.04 (0.03)	NA

HDD = Heavy-duty diesel.
LDD = Light-duty diesel.
NA = Data not available.

[a]From National Research Council (1982), except as indicated.
[b]From Sigsby et al. (1987).
[c]From Bailey et al. (1990), except as indicated.
[d]From Westerholm et al. (1991).
[e]From Smith (1989) and Smith and Paskind (1989); four-cycle FTP test, 1986 Mercedes Benz.
[f]From Hampton et al. (1983), data from Allegheny Mountain Tunnel.
[g]From Hampton et al. (1983), no differentiation between vehicles with and without catalyst.
[h]From Hampton et al. (1983).
[i]From Schuetzle and Frazier (1986).

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 noncatalyst gasoline vehicles shown in Table 2-3 are averaged from 25 in-use passenger cars, representing late 1980s vehicles in intensive use in the United Kingdom (U.K.) (Bailey et al., 1990). The vehicles were driven "as received" and fueled by leaded premium-grade gasoline, obtained locally from a single source. They were driven on five routes chosen to cover the normal range of U.K. driving speeds and conditions, and the exhaust samples were taken using a miniaturized constant-volume exhaust gas sampler. To allow direct comparison, the urban roadway data (≈13.5 mph average speed) are given in Table 2-3. However, it has to be pointed out that hydrocarbon emission rates are highly dependent on driving speeds; in general, THC emission rate expressed in grams per traveled distance decreases as the driving speed increases, but the individual hydrocarbons display various patterns, which relate to their origin. The gasoline components (hydrocarbons with carbon number $C \geq 4$) are present in highest proportion at low speeds, whereas at higher speeds these components are more efficiently used and the proportion of combustion-derived products increases.

The data on LDD emissions are from the National Research Council report (1982) and from studies (Smith, 1989; Smith and Paskind, 1989) concerning the evaluation of particle trap efficiencies for two diesel passenger cars (a 1986 Mercedes Benz and a Volkswagen prototype Jetta). However, little has been published recently; most of the available data are from the late 1970s and early 1980s.

Quantitative data on emissions from heavy-duty vehicles are relatively sparse and are generally expressed in terms of grams per unit work performed by HDD engines. Most of the data on HDD vehicle emission rates expressed in terms of grams per traveled distance were obtained from tunnel field experiments, in particular from the Allegheny and Tuscarora Mountain Tunnel experiments (Hampton et al., 1982, 1983). Unfortunately, because of the sampling method selected, no quantitative data on hydrocarbons with carbon number $C < 8$ could be obtained.

Recently, exhaust emissions from a HDD truck (Scania 143H, equipped with a turbo-charged Scania DSC 1403 diesel engine) were characterized chemically and tested for mutagenicity during transient driving conditions on a chassis dynamometer (Westerholm et al., 1991). Table 2-3 gives selected data from this study.

As can be seen from Table 2-3, 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. 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 evaporative and refueling emissions will result in an increase in the amount of reactive material released.

Table 2-3 lists the emission rates for exhaust pipe emissions only. Currently, fuel evaporation (e.g., from fuel lines and carburetors) accounts for 30% to 60% of the total hydrocarbon emissions from passenger gasoline vehicles with and without catalytic converters (International Agency for Research on Cancer, 1989). Under ambient conditions, the vapor pressure of most diesel fuels in current use is so low that emissions resulting from evaporation are not significant. However, for both diesel and gasoline engine emissions, methane, ethane, ethylene, acetylene, propane, and propylene originate strictly from tailpipe emissions, as evidenced by the fact that the lowest molecular weight components of gasoline are normally hydrocarbons with carbon number C_4.

Differences in the quantity of emitted material between vehicles of the same category are very significant, and such differences arise from many factors of engine 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, dynamometer or road measurements, etc.) make the comparison of the data given in Table 2-3 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, whereas the heavier alkanes ($>C_{10}$) are more characteristic of diesel emissions (Hampton et al., 1983; Carey and Cohen, 1980).

2.3.2. Particulate Emissions

2.3.2.1. *Diesel Particulate Matter*

Diesel exhaust particles are aggregates of spherical primary particles, and 75% to 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. The size distribution is important, because transport of the particles in the atmosphere and deposition in the human respiratory tract depend essentially on aerodynamic diameter (see Chapter 4 for discussion of deposition).

Diesel particulate matter is generally defined as any material that is collected on a filtering medium at a temperature of 52°C or less after dilution of the raw exhaust. In general, diesel engines produce more particulate emissions than do gasoline engines with or without catalytic converters. The main constituent of diesel particles is carbon, which accounts for ≈80%

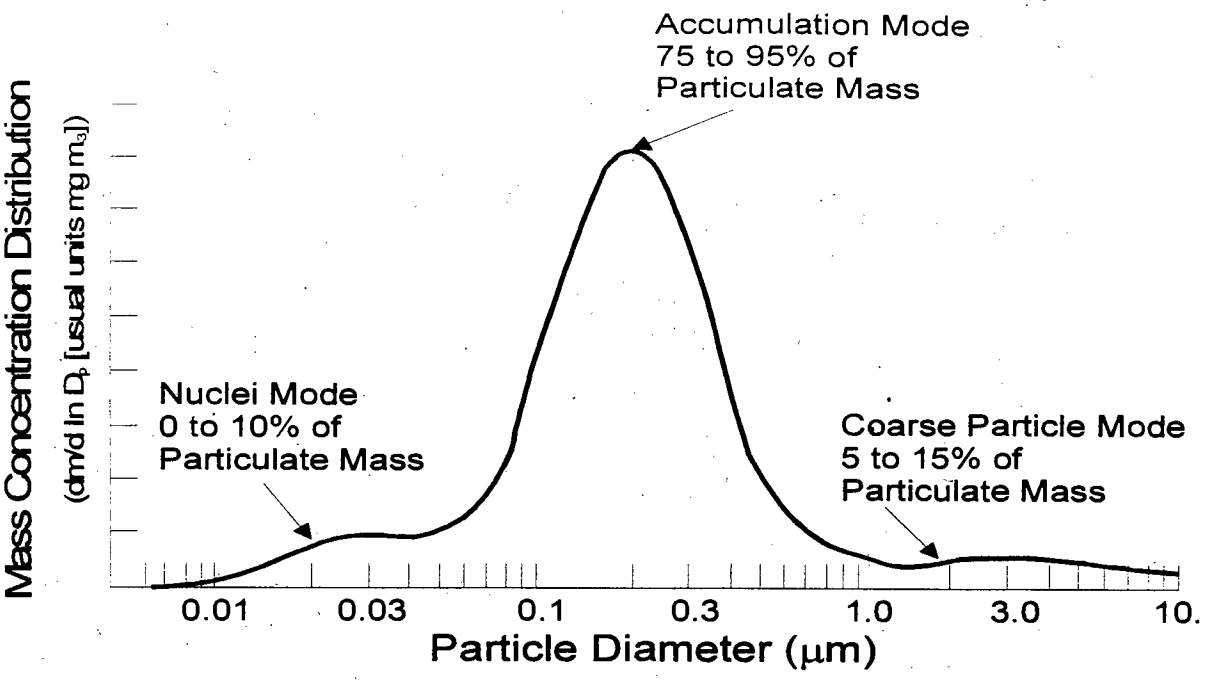


Figure 2-1. Typical size distribution of diesel exhaust particles.

Source: National Research Council (1982).

of total particle mass. Approximately 70% of this so-called total carbon (TC) occurs in the form of elemental carbon (EC); the rest is in the form of organic compounds and is called organic carbon (OC). Table 2-4 compares the emission rates of particulate matter and its distribution between TC and OC for HDD, LDD, and gasoline engines. Data on HDD, LDD, and gasoline engines without catalytic converters are mostly from the survey of 13 HDD vehicles, 19 LDD vehicles, and 22 spark-ignition vehicles in intensive use in Sydney, Australia (Williams et al., 1989a,b). The vehicles were tested on a dynamometer using several test procedures, but Table 2-4 lists the data from the ADR 37 cycle (for spark-ignition and LDD vehicles) and from the modified ADR 36 cycle (for HDD vehicles; multimode steady-state procedure). In addition, data from a heavy-duty truck (Scania 143H), tested on a chassis dynamometer using transient driving conditions (Westerholm et al., 1991), are also given in Table 2-4.

Because the dynamometer studies are not fully representative of the road conditions, the data obtained from the field experiments in two highway tunnels, the Allegheny and Tuscarora Mountain Tunnels of the Pennsylvania Turnpike (Pierson and Brachaczek, 1983b; Szkarlat and Japar, 1983), are also given in Table 2-4 for comparison. Table 2-5 lists the particle-phase and

Table 2-4. Particulate matter emission rates and their distribution between total carbon (TC) and organic carbon (OC) for heavy-duty diesel (HDD), light-duty diesel (LDD), and gasoline engines

Emission	HDD	LDD	Noncatalyst	Catalyst
Particulate matter in g/mi (g/km)	3.2 (2)[a] 1.4 (0.87)[d] 0.99 (0.62)[e]	0.6 (0.37)[a]	0.1 (0.07)[b] 0.04 (0.025)[d]	0.02 (0.01)[c]
TC (% w/w)	78[a]	80[a]	31[b] 67[f]	NA
OC (% of TC)	58[a] 30[d]	36[a]	87[b]	NA

[a]From Williams et al. (1989b).
[b]From Williams et al. (1989a).
[c]From Schuetzle and Frazier (1986).
[d]From Pierson and Brachaczek (1983b).
[e]From Westerholm et al. (1991).
[f]From Szkarlat and Japar (1983).
NA = Not available.

gaseous-phase emissions of diesel exhaust, along with their atmospheric reaction products (discussed in Section 2.4).

2.3.2.2. *Particulate-Phase Inorganics*

Organic and elemental carbon account for ≈80% of the total particulate matter mass. The remaining 20% is composed of sulfate (mainly H_2SO_4) (Pierson and Brachaczek, 1983b) and some inorganic additives and adventitious components of fuel and motor oil. Table 2-6 gives the average compositions of inorganic constituents of airborne particulate matter associated with vehicles on the road (from Pierson and Brachaczek, 1983b). 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).

2.3.2.3. *Particulate-Phase Organic Compounds*

Carbonaceous, diesel-emitted particles have high specific surface areas of 30 to 50 m^2/g (Frey and Corn, 1967). Because of 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 Section 2.1).

Table 2-5. Particle phase and gaseous phase emissions from diesel exhaust, and their atmospheric reaction products

Emission component	Atmospheric reaction products
Particle-phase emissions	
Elemental carbon	—
Inorganic sulfate	—
Hydrocarbons (C_{14}-C_{35})	Little information; possibly aldehydes, ketones, and alkyl nitrates
PAHs (≥4 rings) (e.g., pyrene, benzo[*a*]pyrene)	Nitro-PAHs (≥4 rings); nitro-PAH lactones
Nitro-PAHs (≥3 rings) (e.g., nitropyrenes)	Hydroxylated-nitro derivatives
Gaseous-phase emissions	
Carbon dioxide	—
Carbon monoxide	—
Oxides of nitrogen	Nitric acid, ozone
Sulfur dioxide	Sulfuric acid
Hydrocarbons	
Alkanes ($\leq C_{18}$)	Aldehydes, alkyl nitrates, ketones
Alkenes ($\leq C_4$) (e.g., 1,3-butadiene)	Aldehydes, ketones
Aldehydes	
Formaldehyde	Carbon monoxide, hydroperoxyl radicals
Higher aldehydes (e.g., acrolein)	Peroxyacyl nitrates
Monocyclic aromatic compounds (e.g., benzene, toluene)	Hydroxylated and hydroxylated-nitro derivatives
PAHs (≤4 rings) (e.g., phenanthrene, fluoranthene)	Nitro-PAHs (≤4 rings)
Nitro-PAHs (2 and 3 rings) (e.g., nitronaphthalenes)	Quinones and hydroxylated-nitro derivatives

Source: Health Effects Institute (1995).

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% to 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 results in a relatively low particle concentration and a higher proportion of organic-associated particles (Dutcher et al., 1984). In addition, recent progress in in-cylinder particulate matter

Table 2-6. Summary of composition and emission rates (in milligrams per kilometer) of airborne particulate matter from on-road vehicles, Tuscarora Mountain Tunnel 1977 experiment

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	0.04 ± 0.6 (0.07 ± 0.11)	1.14 ± 0.16 (0.13 ± 0.02)
C	34 ± 21 (67 ± 42)	725 ± 117 (84 ± 14)
N	1.1 ± 0.8 (2 ± 2)	16 ± 2 (1.9 ± 0.3)
Na[c]	0.09 ± 0.37 (0.2 ± 0.7)	6.6 ± 1.0 (0.8 ± 0.1)
Mg[c]	0.7 ± 0.3 (1.3 ± 0.6)	8 ± 1 (0.9 ± 0.15)
Al[c]	0.2 ± 0.5 (0.3 ± 0.9)	8.5 ± 1 (1.0 ± 0.2)
Si[d]	0.5 ± 0.7 (1.0 ± 1.3)	14 ± 2 (1.6 ± 0.2)
P[e]	0.07 ± 0.06 (0.13 ± 0.11)	1.3 ± 0.2 (0.15 ± 0.02)
S[SO_4^{-2}]	0.4[3.4 ± 0.9] (0.9[7 ± 3])	23[42 ± 5] (2.7[4.9 ± 0.9])
Cl[f]	0.8 ± 0.4 (1.6 ± 0.8)	0 (0)
K[d]	0.17 ± 0.08 (0.3 ± 0.2)	1.5 ± 0.2 (0.17 ± 0.03)
Ca[c]	1.3 ± 0.3 (2.5 ± 0.7)	5.8 ± 1.4 (0.7 ± 0.2)
Ti[d]	0.006 ± 0.01 (0.01 ± 0.02)	0.12 ± 0.03 (0.014 ± 0.004)
Mn[c,f]	0.08 ± 0.01 (0.16 ± 0.025)	0.34 ± 0.04 (0.04 ± 0.004)
Fe[c]	0.32 ± 0.32 (0.6 ± 0.6)	5.0 ± 0.9 (0.6 ± 0.1)
Cu	0.04 ± 0.02 (0.07 ± 0.03)	0.22 ± 0.09 (0.025 ± 0.01)
Zn[e]	0.04 ± 0.04 (0.08 ± 0.08)	1.4 ± 0.1 (0.16 ± 0.1)
Br[f]	5.75 ± 0.45 (11.2 ± 0.9)	0 (0)
Ba[f]	0.03 ± 0.01 (0.07 ± 0.02)	0.66 ± 0.03 (0.08 ± 0.033)
Pb[f]	12.4 ± 1.6 (24)	11.5 ± 3 (1.3 ± 0.3)

[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 motor oil.
[f]Attributable to fuel additives.

Source: Pierson and Brachaczek, 1983b.

control has been most effective in reducing the elemental carbon fraction of the particulate matter, so that the organic carbon fraction now accounts for a much larger share than it had previously.

The extractable portion of total carbon, although commonly used as a measure of organic compound content, is not totally equivalent to the OC fraction, as measured by the thermal-optical carbon analysis technique (Japar et al., 1984). The average ratio of OC to extractable mass was shown to be 0.70 ± 0.05, when toluene/propanol-1 mixture was used as an extraction

solvent. and this ratio was probably the result of the presence of both oxygenated organic compounds and inorganic sulfates in the extracted mass.

2.3.2.3.1. ***Extraction and fractionation techniques.*** A variety of solvents and extraction techniques have been used in the past for the separation of organic compounds from diesel particles (Levsen, 1988). 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 best recovery of PAHs, as determined by ^{14}C-B[*a*]P (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.

2.3.2.3.2. ***Chemical composition.*** Table 2-7 lists the general classes of extractable organic compounds identified in POM from combustion emissions, including diesel emissions.

Liquid chromatography methods usually divide the complex environmental mixtures of organic compounds into nonpolar, moderately polar, and polar fractions. This separation is

Table 2-7. Classes of organic compounds identified in particulate-phase combustion emissions

Hydrocarbons
Derivatives[a] of hydrocarbons
Polycyclic aromatic hydrocarbons (PAHs)
Derivatives of PAH
Multifunctional derivatives of PAH
Heterocyclic compounds
Derivatives of heterocyclics
Multifunctional derivatives of heterocyclic compounds

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

Source: Adapted from Schuetzle (1988).

achieved by using specific solvents (or solvent mixtures) for the elution of compounds from chromatographic columns. Schuetzle and co-workers (1985) proposed that standard chemical compounds be selected for establishing reference points for the fractionation of diesel 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 diesel engine emissions, ≈57% of the extracted organic 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_{40}, with a carbon number maximum at C_{22} to C_{26} (Black and High, 1979; Pierson et al., 1983). Polycyclic aromatic hydrocarbons 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 PAHs. 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. More than 90% of the mass and 70% to 100% of the Ames *S. typhimurium*-active material injected onto the column have been recovered (Schuetzle et al., 1985).

2.3.2.3.3. ***Polycyclic aromatic hydrocarbons.*** Particle-bound PAHs and their derivatives (mainly nitrated PAH) attracted considerable attention relatively early because of their mutagenic and, in some cases, carcinogenic properties (see, for example, 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 ultraviolet or fluorescence detection (Levsen, 1988). Table 2-8 lists the PAHs and thioarenes identified and quantified by GC/MS in three LDD 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 2-9 compares the emission rates of several representative PAHs from HDD, LDD, and gasoline (with and without catalytic converter) engines.

2.3.2.3.4. ***Nitrated polycyclic aromatic hydrocarbons.*** Nitro-PAHs (nitroarenes) have been shown to be present in diesel particulate extracts, though in much lower concentration than the parent PAHs (Schuetzle et al., 1981, 1982; Paputa-Peck et al., 1983). Because many nitroarenes

Table 2-8. Polycyclic aromatic hydrocarbons identified and quantified in extracts of diesel particles

Compound[a,b]	Molecular weight	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
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 gas chromatography retention times.
[b]Isomeric alkyl derivatives are not listed separately.
[c]Concentration range as found in the particulate extracts of three Volkswagen 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.
Source: Tong and Karasek, 1984.

Table 2-9. Emission rates of particle-bound polycyclic aromatic hydrocarbons (PAHs) (in milligrams per kilometer) from HDD, LDD, and gasoline engines

			Gasoline cars	
PAH	HDD	LDD	Noncatalyst	Catalyst
Pyrene	17.6 (11)[a]	66 (42)[b]	45 (28)[c]	7 (4.4)[d]
Fluoranthene	27.2 (17)[a]	50 (31)[b]	32 (20)[c]	5 (3.1)[d]
Benzo[*a*]pyrene	<0.1 (0.06)[a]	1 (0.6)[d]	3.2 (2)[c]	0.4 (0.25)[d]
	2.3 (1.4)[e]	ND[b]		
Benzo[*e*]pyrene	0.24 (.15)[a]	3 (1.9)[d]	4.8 (3)[c]	0.4 (0.25)[d]

[a]From Westerholm et al. (1991).
[b]From Smith (1989), four-cycle FTP test, 1986 Mercedes Benz.
[c]From Alsberg et al. (1985).
[d]From Schuetzle and Frazier (1986).
[e]From Dietzman et al. (1980), averaged value for four different engines.

ND = None detected.

are potent direct-acting (e.g., without metabolic activation) mutagens in the Ames assay using *S. typhimurium* strains (Rosenkranz and Mermelstein, 1983), the analysis of nitro-PAHs in diesel POM attracted considerable attention in the early 1980s.

Numerous nitro-PAHs were identified in LDD particulate extracts using capillary GC with thermionic nitrogen-phosphorus detector (NPD) (Paputa-Peck et al., 1983). Positive isomer identification for 16 nitro-PAHs 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, 5- and 8-nitroquinoline. Forty-five additional nitro-PAHs were tentatively identified in this diesel particulate extract (Paputa-Peck et al., 1983).

The concentration of nitro-PAHs 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 2-10 gives the approximate concentrations of several more abundant nitro-PAHs in LDD particulate extracts.

2.3.2.3.5. ***Oxygenated polycyclic aromatic hydrocarbons.*** The moderately polar fraction of diesel particulate extract contains a variety of oxy-PAHs (in particular, aldehydes, ketones, quinones, and acid anhydrides) in much higher amounts than nitro-PAHs, which elute in the

Table 2-10. Concentrations of nitro-polycyclic aromatic hydrocarbons identified in a light-duty diesel (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).

same fraction. Oxy-PAHs are nonmutagenic or very weakly mutagenic; this explains the relatively low interest in this group of compounds. The most detailed study of oxy-PAHs was published by Schuetzle et al. (1981), who identified more than 100 compounds. A large number of oxy-PAHs in the molecular weight range of 182 to 272 were also identified by Tong et al. (1984). The main components identified by Tong and co-workers in particulate matter extracts of three LDD cars (Volkswagens) 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 to 300 μg/g particles. Some of these oxy-PAHs are formed during sampling (Levsen, 1988).

2.3.2.3.6. ***Polar polycylic aromatic hydrocarbon derivatives.*** According to Schuetzle et al. (1985), although 65% to 75% of the directly acting mutagenicity (as tested by Ames *S. typhimurium* assay) for LDD particulate extracts is associated with the fraction of moderate

polarity, more than 65% of the mutagenic activity for HDD particulate extract is concentrated in the most polar fraction. However, because of the serious analytical difficulties, only preliminary data exist on the identification of compounds that are responsible for the mutagenic activity of this fraction (so-called "polar mutagens"). Schuetzle and co-workers (1985) employed the concept of "bioassay directed chemical analysis" (see Section 2.6) for the isolation and identification of polar PAH derivatives from the extracts of HDD particulate matter (National Institute of Standards and Technology [NIST] standard reference material SRM 1650). Several hydroxynitro-PAHs, hydroxy-PAHs, and nitrated heterocyclic compounds were tentatively identified in the polar fraction. It has to be noted, however, that NIST SRM 1650 was not intended to be representative of HDD engines but was a material made available to investigators for the purpose of methods development.

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 MS, using electron impact (EI) and negative ion chemical ionization (NICI) techniques. In addition, direct-probe EI and NICI-MS analyses were performed. More than 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 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.

2.3.3. Gaseous-/Particulate-Phase Emission Partitioning of Polycyclic Aromatic Hydrocarbons

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 2-11 gives the vapor pressures at 25 °C of some representative PAHs ranging from naphthalene to benzo[*a*]pyrene.

The factor of $\approx 10^7$ 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, whereas B[*a*]P, other five-ring PAHs, and higher-ring PAHs are predominantly adsorbed on particles. The intermediate three- and four-ring PAHs are distributed between the two phases.

However, the vapor pressures of these intermediate PAHs can be significantly reduced by their adsorption on various types of surfaces. Because of this phenomenon, the amount and type

Table 2-11. Vapor pressures at 25°C for a series of polycyclic aromatic hydrocarbons[a] (PAHs)

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).

of particulate matter present play 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 high-volume filter or cascade impactor contributes to volatilization of the three- to five-ring PAHs, 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 with the high-volume 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 high-volume 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 2-2. As discussed in the preceding text, the "blow-off" from the filter precludes 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 PAHs less volatile than pyrene were

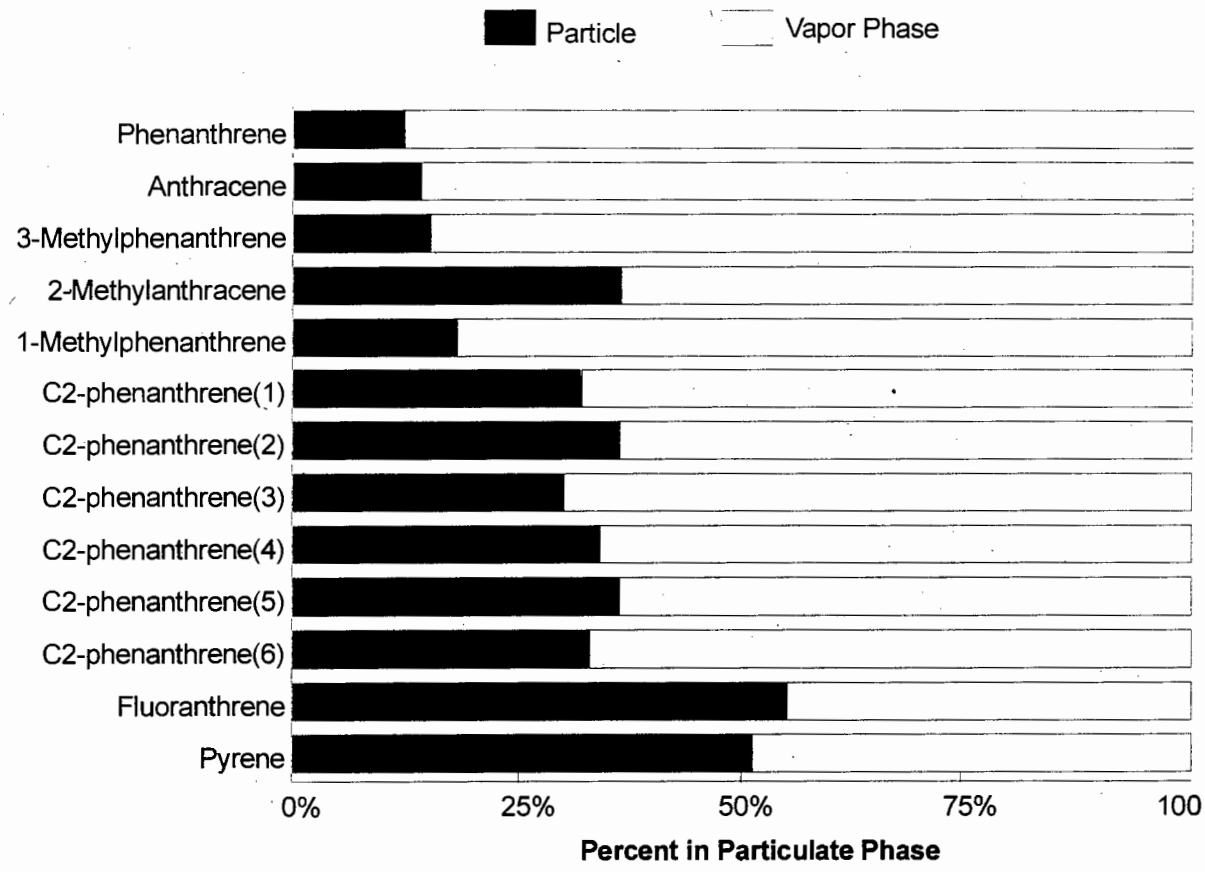


Figure 2-2. Vapor/particle phase polycyclic aromatic hydrocarbon distribution in samples collected in Baltimore Harbor Tunnel.

Source: Benner et al. (1989).

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 2 (Benner et al., 1989).

2.4. ATMOSPHERIC TRANSFORMATIONS OF PRIMARY DIESEL EMISSIONS

2.4.1. Long-Range Transport and Fate of Primary Diesel Emissions

Once released into the atmosphere, primary diesel emissions (or any other direct emissions) are subject 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 with 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 in Figure 2-3.

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 $\approx 10^3$ is more realistic under roadway conditions. This discrepancy leads to slightly different particle size distributions under real driving conditions than those predicted from laboratory data (Kittelson and Dolan, 1980); for example, because of 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 traveled by gaseous- and particulate-phase

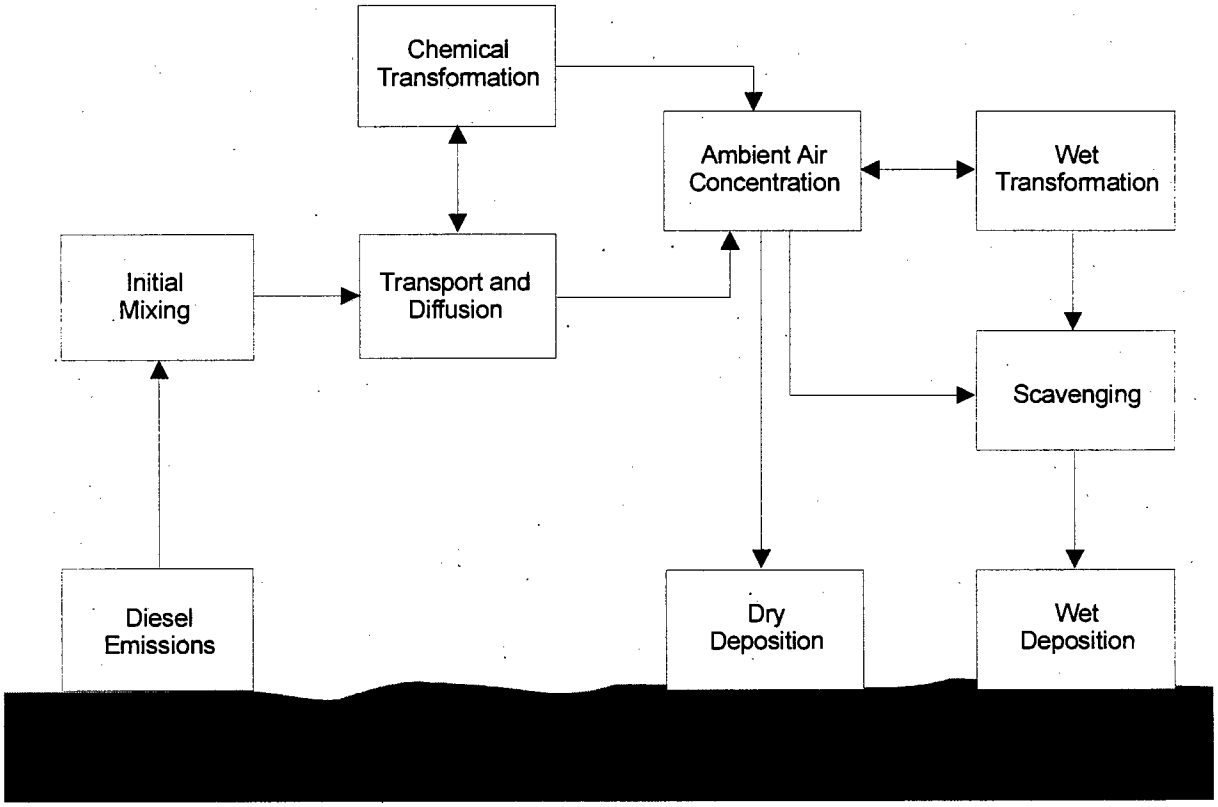


Figure 2-3. Diesel-derived pollutants: emission-to-deposition atmospheric cycle.

Source: Schroeder and Lane (1988).

pollutants depends on the amount of time a specific pollutant resides in the atmosphere and is available for dispersion (Schroeder and Lane, 1988). As primary diesel emissions are a very complex mixture containing thousands of organic and inorganic constituents in the gas and particulate phases that have different chemical reactivities, they are removed by dry and wet deposition processes at different rates. The 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, because these lifetimes determine the geographic extent of the influence of these emissions.

Anthropogenic 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 (Galloway and Whelpdale, 1980; Lowenthal and Rahn, 1985). The organic pollutants, particularly those adsorbed on carbonaceous particles, are also subjected to long-range transport. Organic compounds, such as PAHs, 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 (Figure 2-1), are removed less efficiently than larger particles by wet and dry deposition and thus have longer atmospheric residence times.

It has been reported (Laflamme and Hites, 1978; Hites et al., 1980) that PAHs 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 PAHs increase with proximity to urban areas. This suggests anthropogenic combustion sources and long-range atmospheric transport of PAHs.

Evidence for long-range transport of PAHs was also reported from the recent measurements of PAHs in Siskiwit Lake, located on a wilderness island in northern Lake Superior (McVeety and Hites, 1988). Because of the lake's remote location, PAHs found in this lake are likely to have originated from atmospheric transport.

Earlier studies by Bjørseth and co-workers (Bjørseth and Olufsen, 1983) showed that PAHs are transported from Great Britain and 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, because the amounts of B[*a*]P 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 because the coronene/B[*a*]P ratios in the samples were lower than those usually found in gasoline and diesel exhaust. However, because of the lack of PAH profiles specifically for combustion engines (or,

as a matter of fact, any other specific tracer), the relative contribution of gasoline and diesel vehicle exhausts to long-range transport of organics could not be determined.

2.4.2. Chemical Transformations

2.4.2.1. *Gas-Phase Reactions*

The following chemical processes contribute to the removal of gas-phase compounds 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, typically during 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;
- Reaction with NO_2 during daytime and nighttime hours; and
- Reaction with gaseous nitric acid (HNO_3) and other species such as nitrous (HNO_2) acid and sulfuric acid (H_2SO_4).

It has been shown (Atkinson et al., 1990) that the N_2O_5 reactions with PAHs 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. Because 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.

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 2-12 gives the calculated atmospheric lifetimes for some selected compounds present in automotive gas-phase emissions as the result of known tropospheric chemical removal reactions (Atkinson, 1988). These lifetimes (i.e., the time for the compound to decay to 1/e or

Table 2-12. Calculated atmospheric lifetimes for gas-phase reactions of selected compounds present in automotive emissions with atmospherically important reactive species

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

[a]For 12-h average concentration of OH radical of 1×10^6 molecule/cm^3.
[b]For 24-h average O_3 concentration of 7×10^{11} molecule/cm^3.
[c]For 12-h average NO_3 concentration of 2×10^8 molecule/cm^3.
[d]For 12-h 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-h 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).

Source: Atkinson (1988) unless noted otherwise.

37% of its original concentration) are calculated from the corresponding measured reaction rate constants and the average ambient concentration of the tropospheric species involved.

Although the individual rate constants are known to a reasonable degree of accuracy (in general, to within a factor of 2), 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 2. For this reason, the calculated lifetimes listed in Table 2-12 are approximate only and are valid for those reactive species concentrations listed in the table footnotes. However, these data permit estimation of the contribution of each of these atmospheric reactions to the overall rates of removal of most pollutants from the atmosphere.

As can be seen from Table 2-12, 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 PAHs with N_2O_5 appears to be of minor significance as a PAH loss process but, as will be discussed in subsequent sections, is more important as a route of formation of mutagenic nitro-PAHs.

2.4.2.1.1. ***Reactions of nitrogen oxides.*** Only the major atmospheric reactions of NO_x 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. Nitric oxide is easily oxidized to NO_2 in 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 2-3) with ozone via Reaction 1:

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

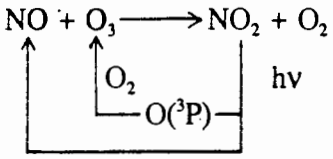
Nitrogen dioxide is photolyzed rapidly at wavelengths of <430 nm:

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

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

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

The photolysis of NO_2 is the only known significant anthropogenic source of ozone in the ambient air and is produced via Reactions 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 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:

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

$$NO_2 + NO_3 \underset{\leftarrow}{\overset{M}{\rightarrow}} N_2O_5$$

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:

$$NO + OH \rightarrow HNO_2$$

$$NO_2 + OH \rightarrow HNO_3$$

2.4.2.1.2. ***Reactions of sulfur dioxide.*** Reaction with OH radical is the dominant SO_2 atmospheric gas-phase reaction process (Stockwell and Calvert, 1983):

$$SO_2 + OH \rightarrow HSO_3$$

followed by the formation of HO_2 radicals and H_2SO_4:

$$HSO_3 + O_2 \rightarrow HO_2 + SO_3$$

$$\downarrow \; H_2O$$

$$H_2SO_4$$

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

2.4.2.1.3. ***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) and Atkinson (1988, 1990).

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

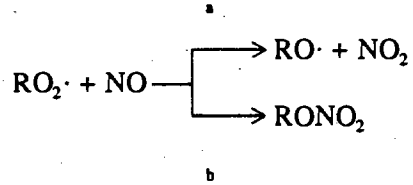
$$RH + OH \rightarrow R^{\bullet} + H_2O$$

$$RH + NO_3 \rightarrow R^{\bullet} + HNO_3$$

Alkyl radical $R^{\bullet}$ reacts with O_2, forming an alkylperoxy radical:

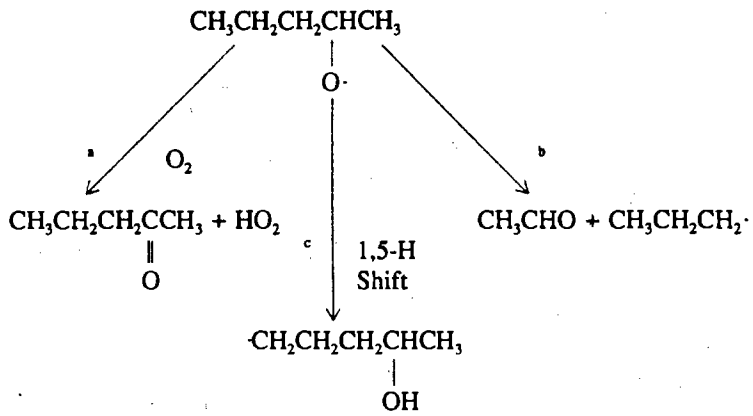
$$R^{\bullet} + O_2 \xrightarrow{M} RO_2^{\bullet}$$

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 ($RO^{\bullet}$), the only significant path for the smaller ($\leq C_4$) radicals and (b) the addition reaction to form stable alkyl nitrates, the significant pathway for larger alkyl peroxy radicals:



Alkoxy radical ($RO^{\bullet}$) 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 in the preceding text; and (c) unimolecular isomerization by 1,4- or 1,5-hydrogen shift (if a hydrogen atom in appropriate position is available), forming α- or β-substituted alkyl radical, which will react with O_2 as discussed in the preceding text.

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.

2.4.2.1.4. ***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). 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 2-13) and proceed by OH radical addition to the double bond.

Because 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):

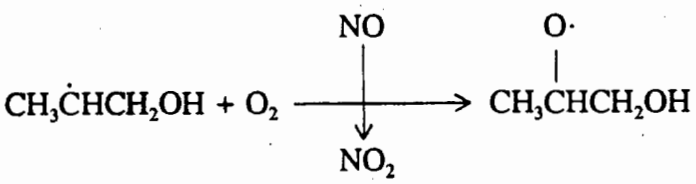


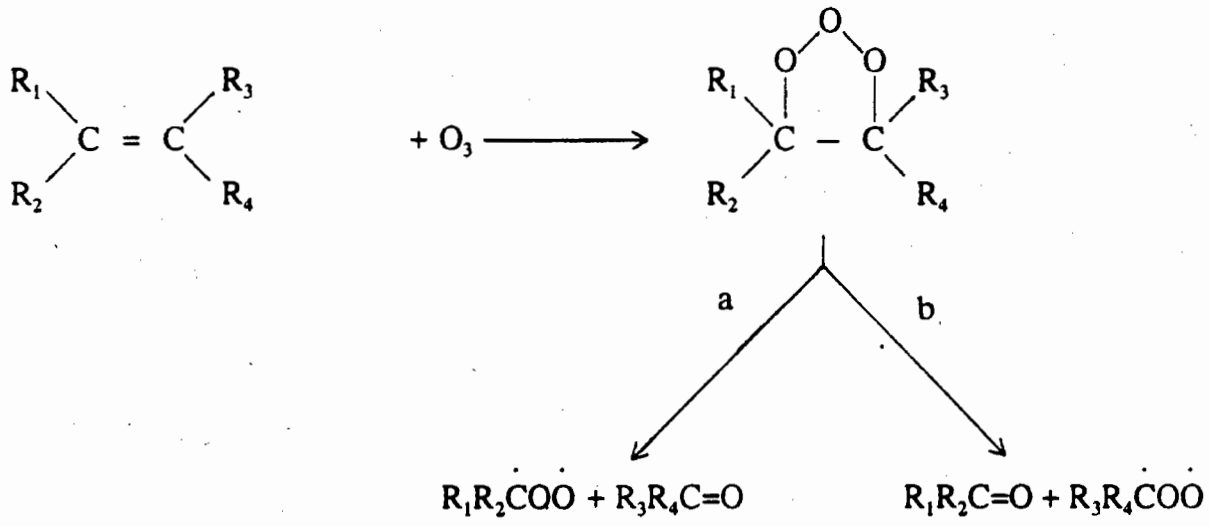
Table 2-3). Similar to the OH radical reaction, this reaction proceeds through NO_3 radical addition to the double bond, followed by reaction with O_2 (Finlayson-Pitts and Pitts, 1986; Atkinson, 1988). Carbonyl compounds are formed as major products, but minor products (possibly dinitrates) are not well defined (Atkinson, 1988).

The reactions of alkenes with ozone compete with the daytime OH radical reaction (Table 2-3). 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:

Table 2-13. Summary of the nitroarenes produced from the gas-phase hydroxyl (OH) radical-initiated and dinitrogen pentoxide (N_2O_5) reactions and electrophilic nitration of polycyclic aromatic hydrocarbons (PAHs)

	Position of nitration (yield) in reaction with		
PAH	**OH**	**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-
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-

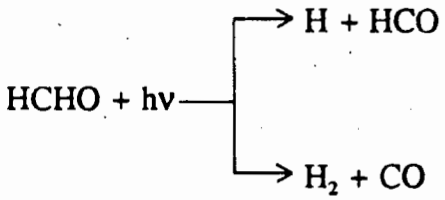
[a]Concurrent NO_3 radical reaction will dominate over N_2O_5 reaction in ambient air.



The importance of pathways (a) and (b) is 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 uncertain (Finlayson-Pitts and Pitts, 1986).

2.4.2.1.5. ***Reactions of oxygen-containing organics.*** Diesel and gasoline vehicle exhausts contain aldehydes and carboxylic acids. The major loss processes for aldehydes involve photolysis and reaction with OH radicals (see Table 2-12). Photolysis is an important loss process for formaldehyde, forming H atom and HCO radical in the first step:



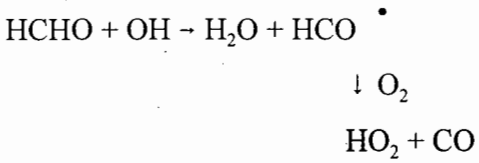
The rapid reaction of H atom and HCO radical with O_2 produces HO_2 radicals:

$$H + O_2 \rightarrow HO_2$$

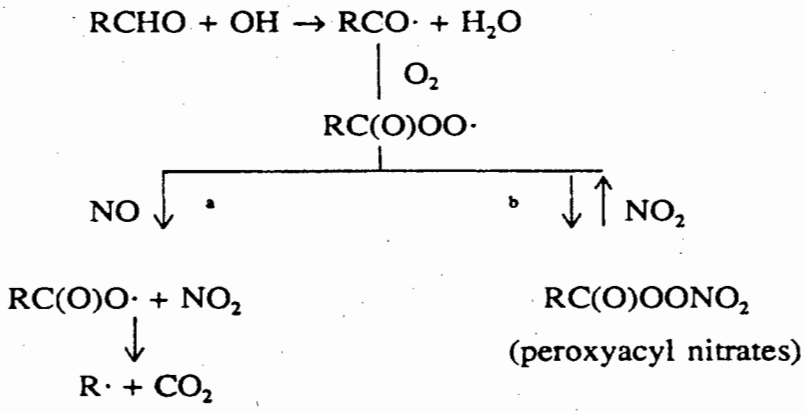
$$HCO + O_2 \rightarrow HO_2 + CO$$

The higher aldehydes also photodissociate, ultimately yielding HO_2 radicals.

The OH radical reaction with formaldehyde yields CO and HO_2:

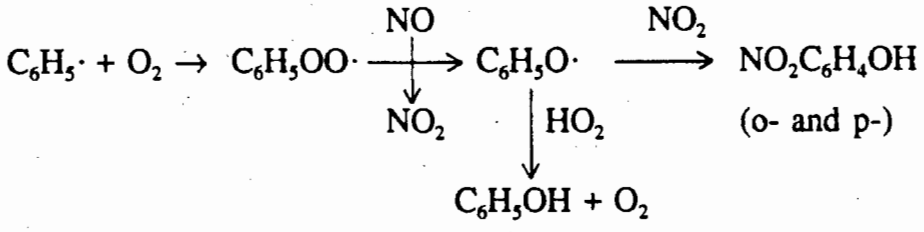


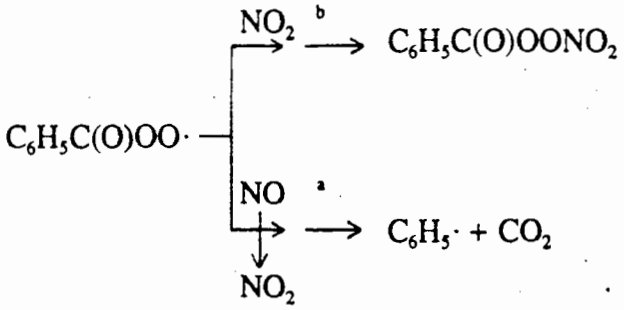
For higher aldehydes, the RCO radicals initially formed add O_2 to yield acylperoxy radical, which can react further with NO (pathway a) and NO_2 (pathway b):



Acetaldehyde forms peroxyacetyl nitrate (PAN), which has been shown to be a direct-acting mutagen toward Ames *S. 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 (Table 2-3). For formic acid, hydrogen atoms are produced (Atkinson, 1988):

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

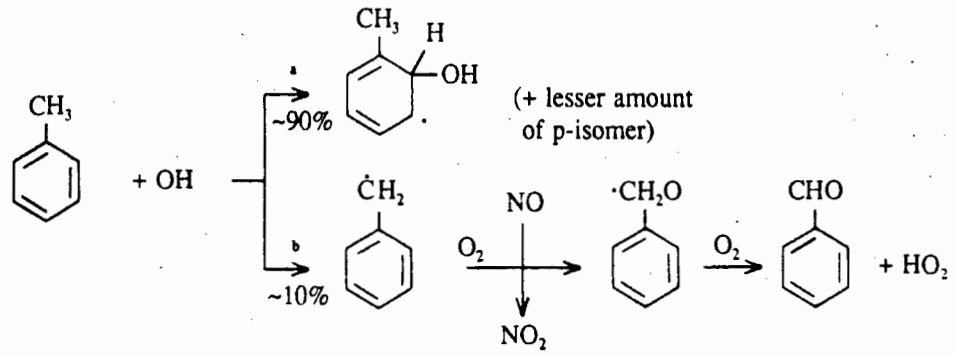
For the higher carboxylic acids, reaction products are currently unknown.

2.4.2.1.6. ***Reactions of monocyclic aromatic compounds.*** The monocyclic aromatic compounds are removed from the atmosphere solely by reactions with OH radicals (see Table 2-3). These reactions proceed by two pathways (Finlayson-Pitts and Pitts, 1986; Atkinson, 1988):

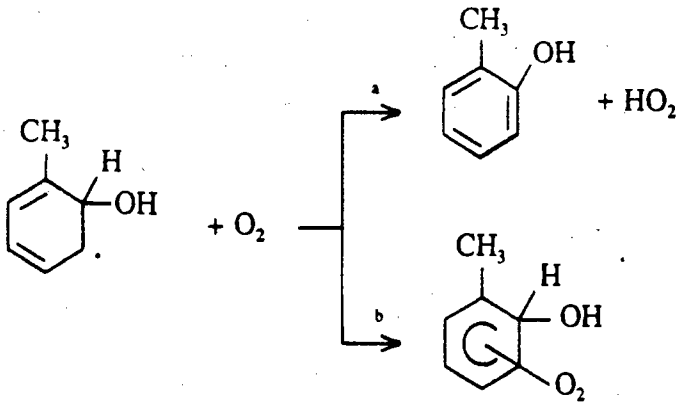
(a) a major pathway by OH radical addition to the aromatic ring, and

(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:

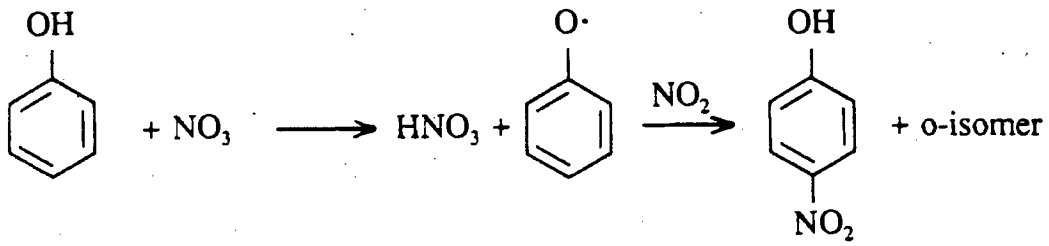


The products arising from the OH radical addition pathway (a) are not well known. Reaction with O_2, again occurring by two pathways, is expected to predominate:



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).

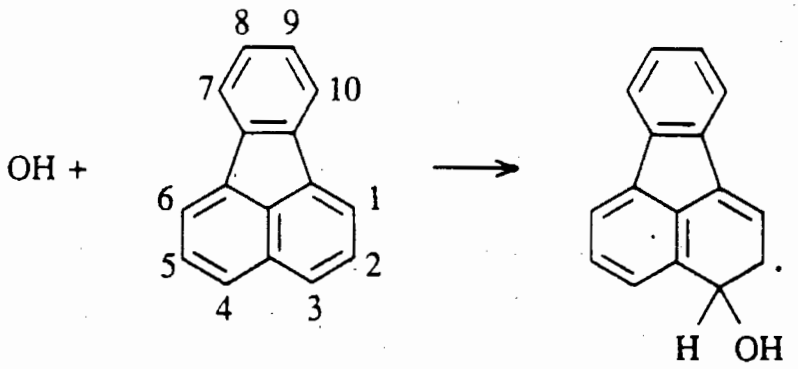
For phenolic compounds, in addition to OH radical reaction (proceeding mainly by initial OH radical addition to the ring), the NO_3 radical reaction that yields nitrophenols appears to be important (Atkinson, 1988):



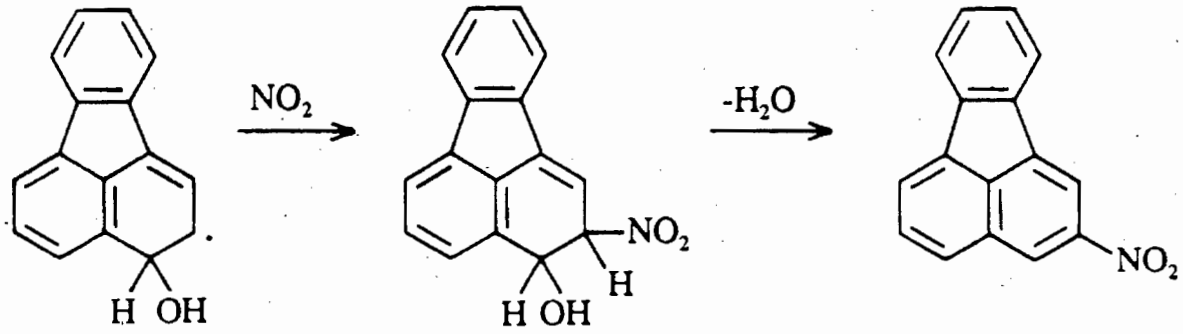
2.4.2.1.7. ***Reactions of polycyclic aromatic compounds.*** As discussed in Section 2.3.4, two- to four-ring PAHs emitted from diesel and spark-ignition engines are distributed between gas and particle phases. For those PAHs present in the gas phase, the reaction with OH radical is predominant, leading to atmospheric lifetimes of a few hours or less (see Table 2-12). The nighttime gas-phase reaction with N_2O_5 is of minor significance as a PAH loss process but (as will be discussed in the following text) 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-methylnaphthalene, acenaphthylene, biphenyl, fluoranthene, pyrene, and acephenanthrylene lead to the formation of nitroarenes (Arey at al., 1986, 1989; Atkinson et al.,

1987, 1990; Zielinska et al., 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 (2-NP) (Pitts et al., 1985a). In contrast, the electrophilic nitration reaction of fluoranthene or pyrene involving the NO_2^+ ion produces mainly 3-nitrofluoranthene (3-NF) from fluoranthene and 1-NP 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 the OH radical reaction but with different yields (Arey et al., 1989; Sweetman et al., 1986; Atkinson et al., 1987, 1990; Zielinska et al., 1986, 1989a). For example, the same 2-NF 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):

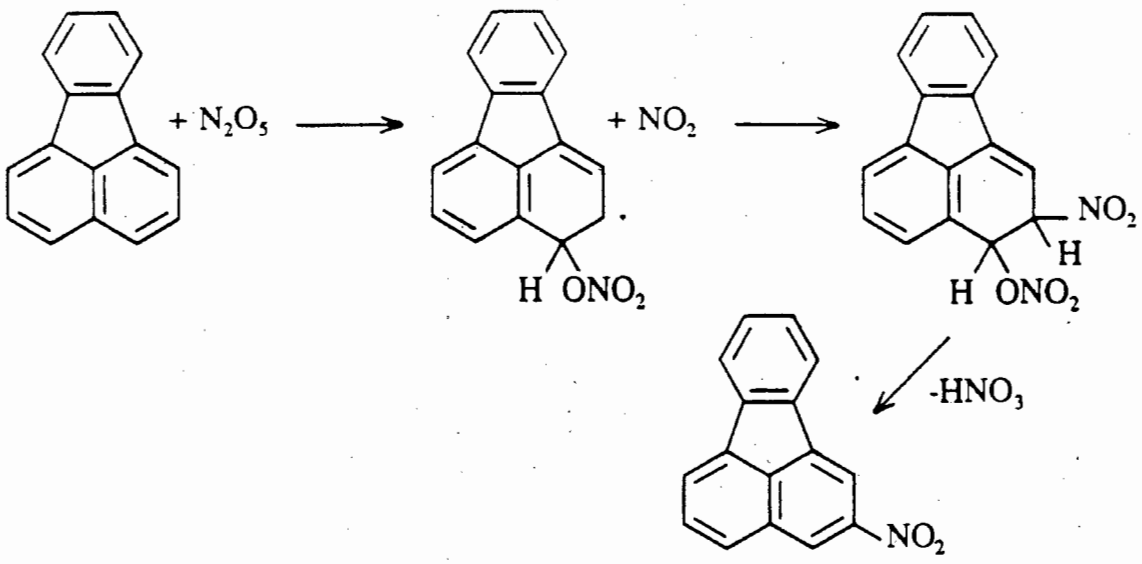


Table 2-13 summarizes the nitroarene product data for OH radical-initiated and N_2O_5 gas-phase reactions with several PAHs studied to date in environmental chambers (Arey et al., 1989; Zielinska et al., 1990). Section 2.5.3 will discuss the fact that, generally, the same nitro-PAH isomers that are formed from OH radical and N_2O_5 reactions are observed in ambient air samples.

2.4.2.2. *Particulate-Phase Reactions*

Organic compounds present in diesel exhaust are partitioned 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,
- Reaction with H_2O_2, and
- Reaction with HNO_3, HNO_2, and H_2SO_4.

However, the atmospheric lifetimes of particle-bound organic compounds are not well known, partly because (1) these chemical processes depend on the nature of the substrate (e.g., Behymer and Hites, 1985, 1988) and (2) 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 PAHs has received much attention since their potential toxicity was first observed. In their recent publication, Behymer and Hites (1988) define two opposite schools of thought on this subject. One says that particle-bound PAHs degrade quickly in the atmosphere with lifetimes as short as a few hours (e.g., Kamens et al., 1988; Nielsen, 1988; Behymer and Hites, 1988). The other says that PAHs degrade slowly, if at all, in the atmosphere and eventually deposit on soil or water. The latter conclusion is supported by the studies of marine and lacustrine sediments (the ultimate environmental sinks of PAHs) that have shown that the relative abundances of PAHs, 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; McVeety and Hites, 1988).

2.4.2.2.1. ***Photooxidation of particulate polycyclic aromatic hydrocarbons.*** Laboratory studies of photolysis of PAHs 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 photodegradation of PAHs depended very much on the nature of the substrate to which they are adsorbed. The dominant factor in the stabilization of PAHs adsorbed on fly ash was the color of the fly ash, which is related to the amount of black carbon present. It appeared that PAHs were stabilized if the black carbon content of the fly ash was greater than ≈5%. On black substrates, half-lives of PAHs studied were on the order of several days (Behymer and Hites, 1988).

Similar conclusions were reached from studies of photolysis of PAHs 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 on the order of 1 h 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.

Atmospheric studies by 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 PAHs, 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.

Because of the limited understanding of the mechanisms of these complex heterogeneous reactions, it is currently impossible to draw any firm conclusion concerning the photostability of particle-bound PAHs in the atmosphere. Because diesel particulate matter contains a relatively high quantity of elemental carbon (see Section 2.3.2.1), it is reasonable to assume that PAHs adsorbed onto these particles should be relatively stable under standard atmospheric conditions. Clearly, additional comprehensive and systematic investigation of adsorbed-phase reactions of PAHs is needed.

2.4.2.2.2. ***Nitration of particulate polycyclic aromatic hydrocarbons under simulated atmospheres.*** Since 1978, when Pitts and co-workers (Pitts et al., 1978) first demonstrated that B[*a*]P deposited on glass-fiber filters exposed to air containing 0.25 ppm of NO_2 with traces of HNO_3 formed nitro-B[*a*]P, numerous studies of the heterogeneous nitration reactions of PAHs adsorbed on a variety of substrates in different simulated atmospheres have been carried out (e.g., Finlayson-Pitts and Pitts, 1986).

PAHs deposited on glass-fiber and Teflon-impregnated glass-fiber filters react with gaseous N_2O_5, yielding their nitro derivatives (Pitts et al., 1985b,c). 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-NP from pyrene, 6-nitro-B[*a*]P from B[*a*]P, and 3-nitroperylene from perylene. For fluoranthene, 3-, 8-, 7-, and 1-NF 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-NF. However, no 2-NF (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, that is, not completely dissociated (Zielinska et al., 1986).

Based on these laboratory studies, it has been proposed that some nitro-PAHs detected in ambient particles may be formed from the reaction of the parent PAH with gaseous copollutants in the atmosphere, during the collection of particulate matter, or both (Pitts et al., 1978, 1985a; Jäger and Hanuš, 1980; Brorström 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 copollutants, relative humidity, etc., have no major impact on PAH nitration reactivities. If these assumptions are valid, 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 air sheds that have high NO_2 and nighttime N_2O_5 concentrations.

The formation of nitro-PAHs during sampling may be an important problem for diesel particulate matter collection because of the presence of NO_2 and HNO_3. However, Schuetzle (1983) concluded that the artifact formation of 1-NP during dilution tube sampling accounts for less than 10% to 20% of the total 1-NP 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 high-volume sampling conditions has been reported to be minimal, at least for the most abundant nitropyrene and nitrofluoranthene isomers (Arey et al., 1988).

2.4.2.2.3. ***Ozonolysis of particulate polycyclic aromatic hydrocarbons.*** Numerous laboratory studies have shown that PAHs deposited on combustion-generated fine particles and on model substrates undergo reaction with O_3 (e.g., Katz et al., 1979; Pitts et al., 1980, 1986; Van Vaeck and Van Cauwenberghe, 1984; Finlayson-Pitts and Pitts, 1986). The dark reaction of several PAHs 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 PAHs, such as B[*a*]P, anthracene, and benz[*a*]anthracene (Katz et al., 1979).

The reaction of PAH deposited on diesel particles with 1.5 ppm O_3 under high-volume sampling conditions has been shown to be relatively fast, and half-lives of the order of 0.5 to 1 h have been reported for most PAHs studied (Van Vaeck and Van Cauwenberghe, 1984). The most reactive PAHs include B[*a*]P, perylene, benz[*a*]anthracene, cyclopenta[*cd*]pyrene, and benzo[*ghi*]perylene. The benzofluoranthene isomers are the least reactive of the PAHs studied, and benzo[*e*]pyrene (B[*e*]P) is less reactive than its isomer B[*a*]P. The implications of this study for the high-volume sampling of ambient POM are important: reaction of PAHs with O_3 could possibly occur under high-volume 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 from 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 high-volume sampling of particles), also have been carried out (Pitts et al., 1986). These experiments showed that significant degradation of more reactive PAHs adsorbed on ambient particulate matter, such as B[*a*]P, pyrene, and benz[*a*]anthracene, may occur in O_3-polluted atmospheres.

2.4.3. Physical Removal Processes

2.4.3.1. *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, surfaces such as 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 of 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).

For particles, the deposition velocities depend on the particle size, exhibiting a minimum for particles of mean diameter of ≈0.1 to ≈1 μm.

2.4.3.2. *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, because many organic compounds are partitioned between the aerosol and vapor phase, processes of both gas and particle scavenging may be important for a given compound (Ligocki et al., 1985a,b; 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 that is associated with particles.

Particle scavenging is a complex process that depends on 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 or growth of the cloud droplet into raindrops.

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 to 10^5 for 0.01 to 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 to 10^6.

Ligocki and co-workers (Ligocki et al., 1985a,b) measured gas- and particle-scavenging ratios for a number of organic compounds, including PAHs and their derivatives. Table 2-14 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 PAHs). Particle scavenging dominates for the alkanes, which are essentially insoluble in water.

The complexity of liquid-phase inorganic acid formation 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 Finlayson-Pitts and Pitts [1986] for more information).

2.5. ATMOSPHERIC CONCENTRATIONS OF PRIMARY DIESEL EMISSIONS AND THEIR TRANSFORMATION PRODUCTS

Most of the data collected on vehicle emissions are from laboratory studies that used dynamometer/dilution tube measurements. The relevance of these measurements to the atmosphere is always a question, because emissions from vehicles on the road have much higher dilution ratios ($\approx 10^3$ vs. 10), are collected at lower temperatures, are composed of a large number of individual vehicle exhausts, have usually experienced longer residence times (seconds to days versus ≈5 s) before collection or measurement, and, as discussed in Section 2.4, have the opportunity to interact with ambient air pollutants (including exhausts of other vehicles and vehicle types).

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

Table 2-14. Mean particle, gas, and overall scavenging ratios for neutral organic compounds[a]

Compound	Mean Ø[b]	Mean W_p	Mean W_g	Mean W[c]	Dominant scav. mech.[d]
Toluene	0.0	0.0	22 ± 5	22 ± 5	g
1,2,4-Trimethylbenzene	0.0	0.0	27 ± 9	27 ± 9	g
Ethylbenzene	0.0	0.0	27 ± 1	127 ± 11	g
m+*p*-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	1,000	g
Fluorene	0.009	15,000	1,500	1,600	g
Phenanthrene + anthracene	0.011	17,000	3,300	3,500	g
9-Fluorenone	0.021	15,000	11,000	11,000	g
Methylphenanthrenes	0.027	13,000	2,500	2,800	g
Fluoranthene	0.053	11,000	6,300	6,600	g
Pyrene	0.071	9,300	5,900	6,100	g
Eicosane	0.14	40,000	NA	5,600	p
9,10-Anthracenedione	0.21	2,400	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	2,600	18,000	7,000	g
Benz[*a*]anthracene	0.75	1,300	12,000	4,000	g
Benzo[*e*]pyrene	0.97	2,000	5,800	2,100	p
Benzo[*a*]pyrene	0.98	1,700	NA	1,700	p
Benzo[*b*+*j*+*k*]fluoranthene	0.98	2,200	7,400	2,300	p
Perylene	1.0	1,800	NA	1,800	p
Tricosane	1.0	22,000	NA	22,000	p
Tetracosane	1.0	16,000	NA	16,000	p
Benzo[*ghi*]perylene	1.0	3,100	NA	3,100	p
Coronene	1.0	5,900	NA	5,900	p

[a]From Ligocki et al. (1985a,b).
[b]Ø = (aerosol)/(vapor + aerosol).
[c]$W = W_pØ + W_g(1-Ø)$.
[d]g = Gas; p = Particle.
NA = Not available.

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-NP 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).

Some data on organic compound concentrations in air sheds heavily affected by motor vehicle emissions (tunnels, roadsides, etc.) are reviewed in the following text.

2.5.1. Volatile Organic Compounds 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 (≈1,050 ± 10% vehicles per hour in each direction) and was classified into four groups: (1) light-duty, including gasoline and diesel vehicles; (2) heavy-duty gasoline; (3) HDD; and (4) motorcycles. Typical distributions were 91.5%, 3.2%, 5.1%, and 0.2%, respectively.

Table 2-15 lists the mean concentrations from four roadsides for selected hydrocarbons and aldehydes, expressed in ppb C and as a percentage contribution of individual hydrocarbons and aldehydes to total nonmethane hydrocarbons (TNMHC) and total aldehydes, respectively.

The roadside VOC distribution was compared with 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 2-3). 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 percentage 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, whereas acetylene was higher than in the dynamometer study. However, noncatalyst vehicles, which constituted 15% of all light-duty vehicles in the roadside study, were not included in the dynamometer study, nor were LDD and HDD vehicles and trucks.

2.5.2. Polycyclic Aromatic Hydrocarbons

Particulate and vapor phase samples were collected from the traffic passing through the Baltimore Harbor Tunnel and analyzed for PAHs and related compounds (Benner et al., 1989). High-volume air samplers equipped with Teflon filters backed by PUF plugs were used for

Table 2-15. Concentrations of individual hydrocarbons and aldehydes measured in the Raleigh, NC, roadside study[a]

Compound	Concentration (ppbC)	Percent contribution[b,c]
Hydrocarbons		
Ethane	16.30	1.81
Ethylene	64.30	7.15
Acetylene	50.90	5.65
Propane	7.90	0.88
Propylene	22.60	2.51
n-Butane	15.80	1.75
1-Butene	5.70	0.64
n-Pentane	25.40	2.82
iso-Pentane	53.00	5.89
Methylcyclopentane	10.40	1.15
Methylcyclohexane	4.70	0.53
n-Decane	3.00	0.33
Benzene	29.00	3.23
Toluene	59.30	6.60
m- and *p*-Xylenes	53.10	5.90
o-Xylene	12.70	1.41
Ethylbenzene	12.00	1.33
TNMHC[d]	900.00	100.00
Total paraffins	369.20	41.00
Total olefins	164.50	18.20
Total aromatics	252.00	28.00
Total unidentified NMHC	63.60	7.10
Aldehydes		
Formaldehyde	6.74	1.05
Acetaldehyde	3.00	18.40
Acrolein	1.20	7.30
Benzaldehyde	2.31	3.88
Total aldehydes	16.38	100.00

[a]From Zweidinger et al. (1988).
[b]Percent based on ppbC.
[c]Percent contribution of individual hydrocarbons to TNMHC and of individual aldehydes to total aldehydes.
[d]TNMHC = Total nonmethane hydrocarbons.

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 tunnel are tabulated in Table 2-16. The ratios of mean particle-phase PAH concentrations to that of B[*e*]P, which is considered to be a nonreactive PAH, are also given in this table.

As can be seen from Table 2-16, 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) because extracts of diesel particulate matter are known to have significant concentrations of methyl and dimethylphenanthrenes (see Table 2-8 and Yu and Hites, 1981).

Factor analysis was applied to the tunnel data in an attempt to identify factors associated with different types of vehicles; two factors were 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 PAHs loaded onto factor 2, which may be associated with the contribution of gasoline-fueled emissions in the tunnel.

Ambient air sampling for PAHs 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, the site was affected by motor vehicle emissions (Atkinson et al., 1988). Samples were collected by means of high-volume samplers equipped with Teflon-impregnated glass-fiber filters backed by PUF plugs. Table 2-17 shows the average (from three daytime and three nighttime samples) concentrations of PAH measured and the ratios of these concentrations to that of B[*e*]P. Unfortunately, no alkylated phenanthrenes were measured.

As can be seen from the comparison of Tables 2-16 and 2-17, the concentrations of all PAHs 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 those of B[*e*]P were also different for the two sites, usually much lower for the Glendora site (except for higher molecular weight PAHs, 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 high-volume sample collections (or both). The generally higher PAH concentrations for nighttime versus 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 O_3

Table 2-16. Particle- and vapor-phase polycyclic aromatic hydrocarbon concentrations for Baltimore Harbor Tunnel samples[a]

Compound	Concentration (ng/m^3)			
	Range, particles	Mean, particles	Mean, vapor[b]	Ratio[c] to B[*e*]P
Phenanthrene	4.3-56	18.0	132	4.3
Anthracene	0.6-12	2.9	18	0.6
3-Methylphenanthrene	3.9-58	13.9	70	3.3
2-Methylphenanthrene	5.3-74	19.0	—	4.6
2-Methylanthracene	0.6-12	3.0	5.3	0.7
9-and 4-Methylphenanthrene 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-Dimethylphenanthrene	4.7-62	14.0	30	3.4
2,7-Dimethylphenanthrene	3.4-38	9.2	16	2.2
1,3-, 2,10-, 3,9-, and 3,10-Dimethyl- and phenanthrene	9.5-119	26.0	61	6.3
1,6- and 2,9-Dimethylphenanthrene	4.5-63	14.0	27	3.3
1,7-Dimethylphenanthrene	3.9-41	10.2	20	2.4
2,3-Dimethylphenanthrene	3.5-41	9.3	16	2.2
Fluoranthene	6.4-69	20.0	16	4.5
Pyrene	9.7-76	27.0	26	6.3
Benzo[*ghi*]fluoranthene	3.2-26	9.6	ND[d]	2.1
Cyclopenta[*cd*]pyrene	7.6-65	20.0	ND	4.6
Benz[*a*]anthracene	1.9-29	7.6	ND	1.5
Chrysene/triphenylene	2.9-47	12.0	ND	2.4
Benzofluoranthenes[*b,j,+k*]	2.2-44	10.6	ND	2.1
Benzo[*e*]pyrene	1.5-19	5.0	ND	1.0
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]Mean ratios to particulate phase B[*e*]P.
[d]None detected.

Table 2-17. Average ambient concentrations of polycyclic aromatic hydrocarbons measured in Glendora, CA[a]

PAH	Total concentration (ng/m^3)	Ratio to B[*e*]P[b]
Phenanthrene[c]	20.0	—
Anthracene[c]	1.0	—
Fluoranthene	5.6 (0.26)[d]	0.27
Pyrene	4.1 (0.35)[d]	0.37
Benzo[*ghi*]-fluoranthene	0.26	0.28
Cyclopenta[*cd*]-pyrene	0.09	0.1
Benz[*a*]anthracene	0.2	0.22
Chrysene/Triphenylene	1.0	1.1
Benzofluoranthenes[*b,j+k*]	1.6	1.7
Benzo[*e*]pyrene	0.94	1.0
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]Ratios of particle-phase PAH to particle-phase B[*e*]P.
[c]Phenanthrene and anthracene were not present on filters, only on PUF plugs.
[d]Fluoranthene and pyrene are distributed between gas and particulate phases; numbers in parentheses represent particle concentrations.

concentrations (which always occurred between 1400 and 1700 hours, Pacific standard time) ranged from 160 to 240 ppb throughout the entire 9 days of the study.

2.5.3. Nitroarene Concentrations in Ambient Air

Diesel particulate matter contains a variety of nitroarenes, with 1-NP being the most abundant among identified nitro-PAHs. The concentration of 1-NP 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 for either diesel or spark-ignition engines.

Several nitroarene measurements were conducted in air sheds heavily affected by motor vehicle emissions (Arey et al., 1987; Atkinson et al., 1988; Zielinska et al., 1989a,b; Ciccioli et al., 1989). For example, ambient particulate matter samples were collected at three sites (Claremont, Torrance, and Glendora) in the Los Angeles Basin; the Claremont and Glendora sites are ≈30 km and ≈20 km northeast, respectively, and the Torrance site is ≈20 km southwest

of downtown Los Angeles (Arey et al., 1987; Atkinson et al., 1988; Zielinska et al., 1989a,b). The sampling was conducted during two summertime periods (Claremont, September 1985, and Glendora, August 1986) and one wintertime period (Torrance, January and February 1986). Table 2-18 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 Table 2-18, 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 heavily affected by motor vehicle emissions. Of the two nitropyrene isomers present, 2-NP, the main nitropyrene isomer formed from the gas-phase OH radical-initiated reaction with pyrene (see Section 2.4.2.1), is sometimes more abundant. The 2-NF was always the most abundant nitroarene observed in ambient particulate matter collected at these three sites (Ciccioli et al., 1989), and this nitrofluoranthene isomer is not present in diesel and gasoline vehicle emissions. The 2-NF is the only nitroarene produced from the gas-phase OH radical-initiated

Table 2-18. Maximum concentrations of nitrofluoranthene (NF) and nitropyrene (NP) isomers observed at three South Coast Air Basin sampling sites

	Concentration (pg/m^3) at		
Nitroarene, collection period	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 hours and nighttime sample from 1800 to 2400 hours on September 13, 1985.
[c]From Atkinson et al. (1988).
[d]Daytime sample collected from 0800 to 2000 hours on August 20, 1986, and nighttime sample from 2000 to 0800 hours on August 20 and 21, 1986.
[e]Daytime sample collected from 0500 to 1700 hours on January 28, 1986, and nighttime sample from 1700 to 0500 hours on January 27 and 28, 1986.
[f]ND = None detected.

N_2O_5 reactions with fluoranthene (see Sections 2.4.2.1 and 2.4.2.2), whereas mainly 3-NF and 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 2-4 compares 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). It is very unlikely that N_2O_5 could have been present during the nighttime winter collections in Torrance, given the high level of NO present at sunset. More likely, a relatively high level of OH radicals was present because of the measured high concentration of HNO_2, which photolyzes to yield OH radicals. This suggests that all isomers observed in Figure 2-4 (lower trace), with the exception of 1-NP, are the product of the OH radical-initiated reactions of the parent PAH. Direct emissions may account for the 1-NP (and 3-NF) 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.)

Although the reaction with OH radicals is the major atmospheric loss process for gas-phase fluoranthene and pyrene (Table 2-12), evidence for atmospheric formation of 2-NF from N_2O_5 reaction with fluoranthene has also been reported (Zielinska et al., 1989b). Because the 2-NF/2-NP yield ratio for N_2O_5 reactions, observed from environmental chamber experiments, is >100, compared to ≈10 for the OH radical reaction (Table 2-4), the high 2-NF/2-NP concentration ratio in ambient samples suggests a contribution from the N_2O_5 reaction with fluoranthene. Figure 2-5 shows a comparison of a wintertime sample collected in Torrance (upper trace) with a summertime sample collected in Claremont (lower trace). The 2-NF/2-NP 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-NF via reaction with N_2O_5 (Zielinska et al., 1989b).

The evidence presented in the preceding text, as well as the observation that 2-NF 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 in Rome, Italy, at a height of 1.5 m above street level, 2-NF and 2-NP were not observed (Ciccioli et al., 1989).

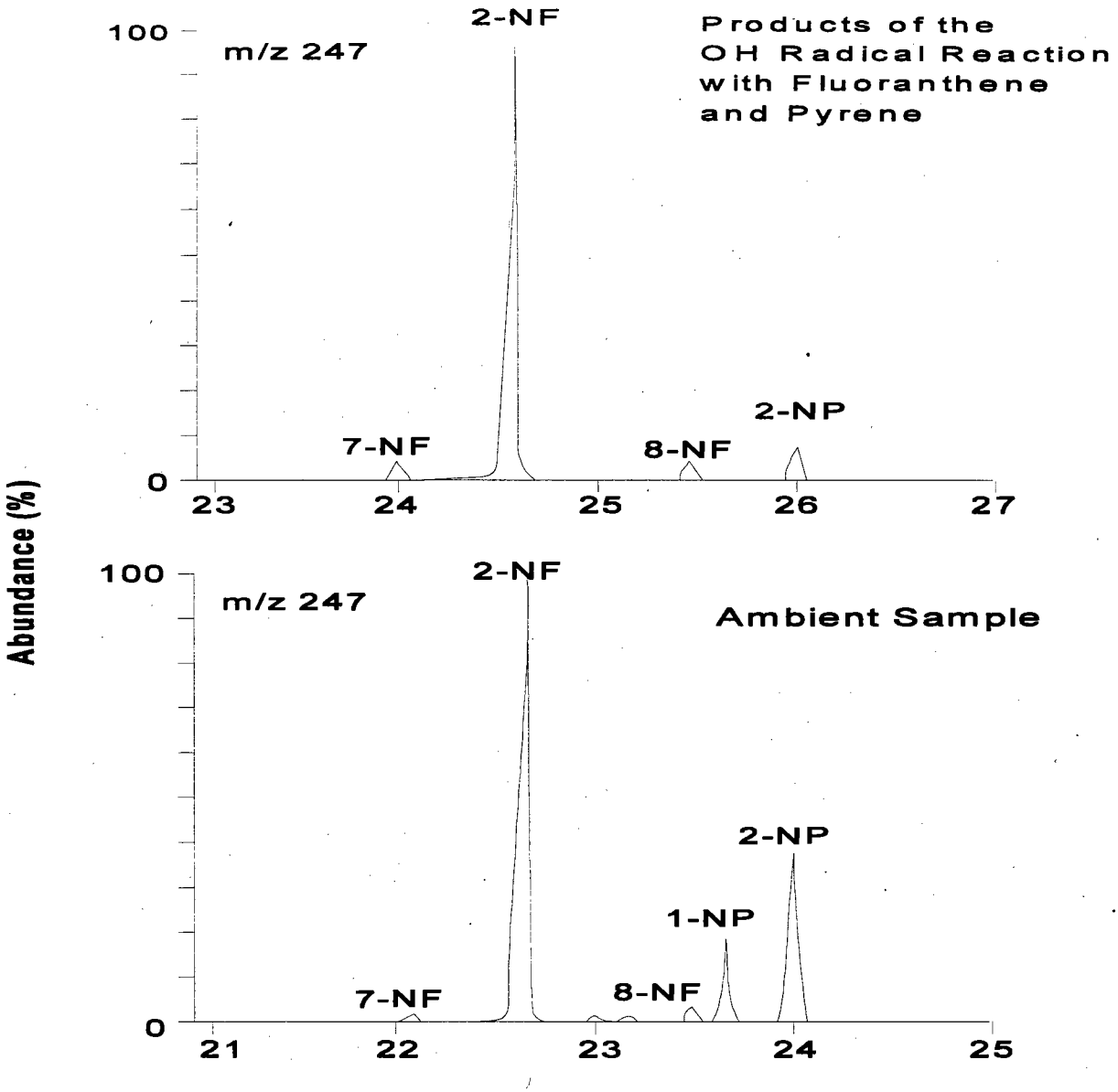


Figure 2-4. Mass chromatograms of the molecular ion of the nitrofluoranthenes (NF) and nitropyrenes (NP) formed from the gas-phase reaction of fluoranthene and pyrene with the OH radicals (top) and present in the ambient particulate sample collected at Torrance, CA (bottom).

Source: Arey et al. (1989).

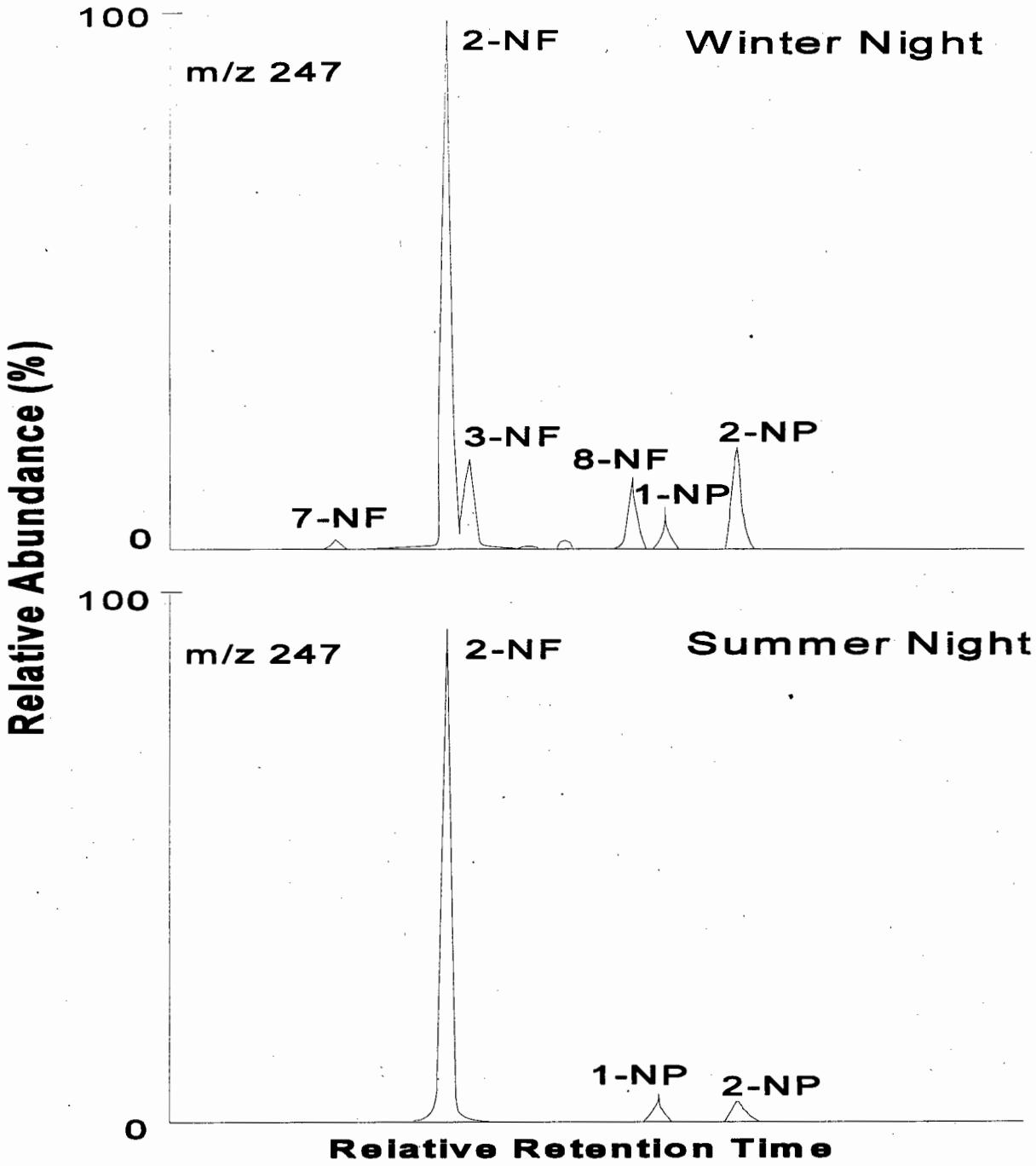


Figure 2-5. **Mass chromatograms of the molecular ion of the nitrofluoranthenes (NF) and nitropyrenes (NP) present in ambient particulate samples collected in Torrance, CA (top), and Claremont, CA (bottom).**

2.6. EXPOSURE PERSPECTIVE

Diesel emissions are complex mixtures containing thousands of organic and inorganic constituents in both gas and particulate phases and with differing chemical reactivities. After entering the atmosphere, they are transported and transformed according to their distinctive characteristics, undergoing physical and chemical changes that may form secondary pollutants more harmful than their predecessors. Thus, a knowledge of diesel emissions at or near their sources is not sufficient to fully assess the impact of these emissions on human health and welfare. However, data on how diesel exhaust contributes to exposure levels for these secondary pollutants are currently lacking.

Determining the amount of diesel exhaust present in the ambient air is also complicated by the difficulty of distinguishing organic compounds and particles that originate in diesel engines from those that originate in gasoline engines or come from other sources. This, too, cannot be accomplished at present because of the lack of a sufficient research base.

Nonoccupational exposure to diesel exhaust is worldwide in urban areas, with lesser exposure in rural areas. Certain working populations are also exposed to higher levels of diesel exhaust than the rest of the population. The level of exposure will differ within geographic areas based on the number and types of diesel engines in the area, as well as atmospheric patterns of dispersal and the location of the individual relative to the emission sources.

While a detailed exposure assessment for DE has not been conducted as part of this study, the following exposure data are provided to give some context for the hazard assessment and dose-response analysis.

Estimates of annual average concentrations of particulate matter in the ambient air, published in Chapter 9 of EPA's Motor Vehicle-Related Air Toxics Study (U.S. EPA, 1993), may be used to generate a crude estimate of the concentration of particulates from diesel exhaust in the ambient air. The Air Toxics study used two approaches to generate exposure estimates. In the first one, DPM (diesel particulate matter) national fleet average emissions factors for 1988 were multiplied by the urban and rural grams-per-meter conversion factors obtained from EPA's hazardous air pollution exposure model (HAP-EM: 1988). Based on this approach, the total concentration of particulates from diesel exhaust in ambient air in urban areas for 1995 was estimated as 2 $\mu g/m^3$; the concentration in rural areas as 0.6 $\mu g/m^3$; and the nationwide average concentration as 1.1 $\mu g/m^3$.

In a second method, using ambient monitoring data, total suspended particulate matter (TSP) for 1990 was determined to equal 48 $\mu g/m^3$. Approximately 5% of total particulate matter is associated with diesel exhaust. Multiplication of the total by the fraction contributed by diesel exhaust, and adjusting for time spent indoors, results in an integrated estimate of 1.5 $\mu g/m^3$ (U.S. EPA, 1993).

Exposure estimates for more highly polluted locations are somewhat greater. Estimated mean concentrations of DPM for Los Angeles were reported to be 2.7 $\mu g/m^3$ (Sienicki and Mago, 1992). McClellan at al. (1986) estimated concentrations on urban freeways and street canyons to be as great as 15 $\mu g/m^3$. These exposure estimates are summarized in Table 2-19.

Recent Cal-EPA (1996) studies show winter period estimates in three California locations for diesel PM_{10} of 4 to 22 $\mu g/m^3$. A broader Cal-EPA analysis shows average ambient outdoor diesel PM_{10} to range from 0.2 to 3.6 $\mu g/m^3$ across 14 California air basins with a population-weighted average of 3.2 $\mu g/m^3$. Concentrations in occupational settings may be higher.

Potentially, diesel exhaust may contribute to levels of a very hazardous pollutant, dioxin. Only scant information is available to quantify this, though some study data are available. Dioxin concentrations were measured in a Baltimore tunnel used only by heavy trucks (unpublished). Measurements were also made of the exhaust from a heavy-duty truck during highway travel. Based on these measurements, estimated dioxin production from all truck diesels was estimated to be only 29 gm versus 5,000 gm from all combustion sources in the United States. It is therefore concluded that DE contribution to dioxin levels in the United States is insignificant.

The changing composition of DE, (i.e., older engines vs. newer technology ones, heavy-duty vs. light-duty, and engines run under varied operating conditions) gives rise to questions about how the health data and the risk assessment findings in this report, which are based on pre-1998 engines, can be applied to present-day engine exhaust emissions and the resulting ambient

Table 2-19. Estimated annual ambient concentrations of diesel exhaust particulate matter ($\mu g/m^3$)

Year	U.S. EPA (1993) Method 1 Rural	U.S. EPA (1993) Method 1 Urban	U.S. EPA (1993) Method 1 National	U.S. EPA (1993) Method 2 National	Sienicki and Mago (1992) Los Angeles	McClellan (1986) Highly Exposed
1986						15.0
1990	1.1	2.0	1.8	1.5	2.7	
1995	0.6	1.2	1.1			
2000	0.4	0.7	0.6			
2010	0.2	0.4	0.4			

exposures. This is a complex question that is not rigorously addressed in this version of the assessment. It is clear that newer technology engines will have somewhat different emission composition (i.e., perhaps reduced NO_x with increased fine particles), not to mention the emission controls, which would reduce certain exhaust components, presumably larger particles. Since particle mass is the surrogate dosimeter used to correlate toxicity with exposure and public health impact, as the particle mass is changed by virtue of new technology or controls, so might the applicability of the health assessment findings in this report. Further analysis of emission changes may be a desirable research pursuit.

2.7. SUMMARY

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

However, many of these data were collected using laboratory dynamometers with selected new vehicles or vehicles well tuned to manufacturers' specifications. The well-controlled conditions of the dynamometer tests have many benefits but do not necessarily represent vehicle emissions under real on-road conditions, and 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 database on mobile-source 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 subject to dispersion and transport and, at the same time, to chemical and physical transformation 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 past two decades of laboratory and ambient experiments and computer modeling, a comprehensive set of data now exists concerning the atmospheric loss processes and transformation of automotive emissions, but knowledge concerning the products of these chemical transformations is still limited. Study is required to determine the products from

the OH radical-initiated reactions of the aromatic and aliphatic hydrocarbons from automobile emissions. The atmospheric transformation products of PAHs 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" that may be used in receptor source apportionment models. The data indicate that it may be possible to use PAHs and/or alkylated PAHs, 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 by using sampling and analytical methods appropriate to receptor modeling.

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CHAPTER 3

NOTE TO READERS

Because Chapter 3 recently has merged with Chapter 2, time did not permit renumbering of the remaining chapters for this draft. Chapters 4 through 12 will be renumbered in the next version.

4. DOSIMETRIC FACTORS

4.1. INTRODUCTION

Diesel engine emissions consist of a complex mixture of gases, vapors, and particles made up of a carbon core with a great variety of organic agents adsorbed to the surface. The tumorigenic response observed in rats exposed to diesel exhaust is the result of pulmonary overloading with diesel soot particles. Data from studies in rats have shown that exposure duration and particle surface area and/or particle mass are relevant dosimetric factors. These same studies have shown that particle-adsorbed organics may not be instrumental in the tumorigenic response in rats. Whether similar tumorigenic mechanisms in humans exposed at low doses are critical dosimetric factors in estimating human risk to diesel exhaust is uncertain. Although assessment of dose-response relationships may permit more advanced extrapolations from high experimental exposure concentrations to ambient levels and from animal test species to humans, the question of mechanistic similarities in a tumorigenic response between rats and humans remains unanswered.

A review of animal carcinogenicity studies (Chapter 7) revealed that the gaseous phase alone failed to induce increases in lung tumors in any of the long-term studies in rats or mice. Because of the very limited positive data for this fraction and because the potential carcinogens likely to be present in this fraction (formaldehyde and acetaldehyde) induce upper respiratory tract tumors, which were not seen in the whole-exhaust studies, the gaseous phase is not considered separately in determining carcinogenic risk of diesel exhaust. Noncancer endpoints examined in these studies (Chapter 5) also were more affected by the whole exhaust compared to the gas phase of the exhaust. Because the tumorigenic effects observed in rats are likely to be a function of particle overload, the dosimetry of particles will be emphasized in this chapter. The dosimetry of particle-adsorbed organics, however, will be included because they may be of greater importance for humans exposed at low concentrations.

With a single exception (Iwai et al., 1986), the tumors reported in the diesel exhaust inhalation studies reviewed in Chapter 7 all occurred in the lungs. However, these findings are questionable because of the relatively small number of animals used and the fact that the lymphomas reported by Iwai et al. (1986) were not observed in other studies where rats were similarly exposed. Because particle overload carcinogenesis occurs under pulmonary overload conditions, dosimetric considerations are limited to the lung.

The insoluble carbon core of the exhaust particle and its persistence in the lung are now recognized as critical factors in the particle overload phenomenon that apparently leads to the tumorigenic response observed in rats. Furthermore, total particle surface area appears to be an

important determinant in the particle overload phenomenon. The contribution of extractable organics has been shown to be inconsequential in this response (this subject is discussed in more detail in Chapter 10). It is, however, important to note that dose determination for inhaled compounds remains problematic among toxicologists (Dahl et al., 1991).

The dosimetric aspects considered will include deposition in the conducting airways and alveolar regions, normal particle clearance mechanisms and rates in both regions, clearance rates during lung overload, elution of organics from the particles, particle transport to extraalveolar sites, and the interrelationships of these factors in determining the target organ dose.

4.2. REGIONAL DEPOSITION OF INHALED PARTICLES

The regional deposition of particulate matter in the respiratory tract is dependent on the interaction of a number of factors, including respiratory tract anatomy (airway dimensions and branching configurations), ventilatory characteristics (breathing mode and rate, ventilatory volumes and capacities), physical processes (diffusion, sedimentation, impaction, and interception), and the physicochemical characteristics (particle size, shape, and density) of the inhaled particles. Regional deposition of particulate material is usually expressed as deposition fraction of the total particles or mass inhaled and may be represented by the ratio of the particles or mass deposited in a specific region to the number or mass of particles inspired. The factors affecting deposition in these various regions and their importance in understanding the fate of inhaled diesel exhaust particulate matter are discussed in the following sections. It is beyond the scope of this document to present a comprehensive account of the complexities of respiratory mechanics, physiology, and toxicology. Where appropriate, the reader is referred to publications that provide a more in-depth treatment of these topics (Weibel, 1963; Brain and Mensah, 1983; Raabe et al., 1988; Stöber et al., 1993).

4.2.1. Physical Processes, Physiological/Anatomical Considerations, and Particle Characteristics

Deposition of particles may occur through several processes or combinations thereof, including diffusion, sedimentation (gravitational settling), interception, electrostatic precipitation, and impaction. It is important to appreciate that these processes are not necessarily independent but may, in some instances, interact with one another such that total deposition in the respiratory tract resulting from these processes may be less than the calculated probabilities for deposition by the individual processes (Raabe, 1982). Depending on the particle size and mass, varying degrees of deposition may occur in the nasopharyngeal, tracheobronchial, and alveolar regions of the respiratory tract.

Upon inhalation of particulate matter such as diesel exhaust, deposition will occur throughout the respiratory tract. Because of high airflow velocities and abrupt directional changes in the nasopharyngeal and tracheobronchial regions, inertial impaction is a primary deposition mechanism (especially for particles ≤ 2.5 μm mass median diameter [MMD]). Although inertial impaction is a prominent process for deposition of larger particles in the tracheobronchial region, it is of minimal significance as a determinant of regional deposition patterns for diesel exhaust particles, with an MMD ≤ 1 μm and small aspect ratio.

Because their MMD is generally ≤ 1 μm, diesel exhaust particles are subject to deposition in the alveolar region. Based on animal data regarding the site of origin of diesel exhaust-induced tumors, particle deposition in the alveolar region may be of greatest concern relative to the carcinogenic potential of diesel particulate matter and/or the adsorbed organics. However, such data for humans are not available. For such small particles, diffusion would be especially prevalent in this region, whereas sedimentation would become less significant, especially for particles of MMD <0.5 μm.

Respiratory tract anatomy and ventilatory characteristics are crucial in determining regional deposition patterns and are also responsible for interspecies and interindividual variability in both deposition of particulate matter and inhaled dose. The variability in size and branching configurations of conducting airways is an important determinant of interspecies variability in deposited dose. Because of the anatomical complexity and variability in ventilatory patterns, a precise categorization of airflow dynamics for a given species or for a specific portion of the respiratory tract is difficult. For more extensive discussions of deposition processes, refer to reviews by Morrow (1966), Raabe (1982), U.S. Environmental Protection Agency (1982), Phalen and Oldham (1983), Lippmann and Schlesinger (1984), Raabe et al. (1988), and Stöber et al. (1993).

Exposure to whole diesel exhaust will also result in inhalation of gas-phase components such as formaldehyde, acrolein, and sulfur dioxide, all of which have been demonstrated to be sensory irritants. It is also known that these irritants affect respiratory rates (Kane and Alarie, 1978, 1979). 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 concentration-dependent response (Alarie, 1966, 1973; Kane and Alarie, 1978). Several studies have also shown that mice appear to be responsive to sensory irritants relative to alteration of respiratory patterns (Alarie, 1973; Kane and Alarie, 1978, 1979). However, only very low levels of these irritants are present in diesel engine exhaust; consequently, their significance in affecting delivered dose through changes in respiration may be small.

4.2.2. Species Variability in Regional Dose

The variability in the anatomy of conducting airways among species results in interspecies variability of inhaled dose. Because different species breathing the same aerosol will not receive the same dose to the respiratory tract, it is generally accepted that exposure concentration is not an accurate description of respiratory tract dose (Brain and Mensah, 1983).

The deposition of inhaled diesel particles in the respiratory tract of humans and mammalian species has been reviewed by Schlesinger (1985). He showed that physiological differences in the breathing mode for humans (nasal or oronasal breathers) and experimental animals (obligatory nose breathers), combined with different airway geometries, resulted in significant differences in lower respiratory tract deposition for larger particles (>1 μm). In particular, a much lower fraction of inhaled larger particles is deposited in the alveolar region of the rat compared with humans. However, relative deposition of the much smaller diesel exhaust particles was not affected as much by the differences among species, as was demonstrated in model calculations by Xu and Yu (1987). These investigators modeled the deposition efficiency of inhaled diesel exhaust particles in rats, hamsters, and humans on the basis of calculations of the models of Schum and Yeh (1980) and Weibel (1963). In Figure 4-1, relative deposition patterns in the lower respiratory tract (trachea = generation 1; alveoli = generation 23) are very similar among hamsters, rats, and humans. Variations in alveolar deposition of diesel exhaust particles over one breathing cycle in these different species were predicted to be within 30% of one another. Xu and Yu (1987) attributed this similarity to the fact that deposition of the submicron diesel particles is dominated by diffusion rather than sedimentation or impaction. Although these data assumed nose-breathing by humans, the results would not be very different for mouth-breathing because of the low filtering capacity of the nose for particles in the 0.1 to 0.5 μm range.

However, for dosimetric calculations and modeling, it would be of much greater importance to consider the actual dose deposited per unit surface area of the respiratory tract rather than the relative deposition efficiencies per lung region. Table 4-1 compares the predicted deposited doses of diesel exhaust particles inhaled in 1 min for the three species, based on the total lung volume, the surface area of all lung airways, or the surface area of the epithelium of the alveolar region only. In Table 4-1, the absolute deposited dose is lower in humans than in the two rodent species as a result of the greater respiratory exchange rate in rodents and smaller size of the rodent lung. Such differences in the absolute deposited dose in relevant target areas are important and have to be considered when extrapolating the results from diesel exhaust exposure studies in animals to humans. The differences are less on a surface area basis than on a lung

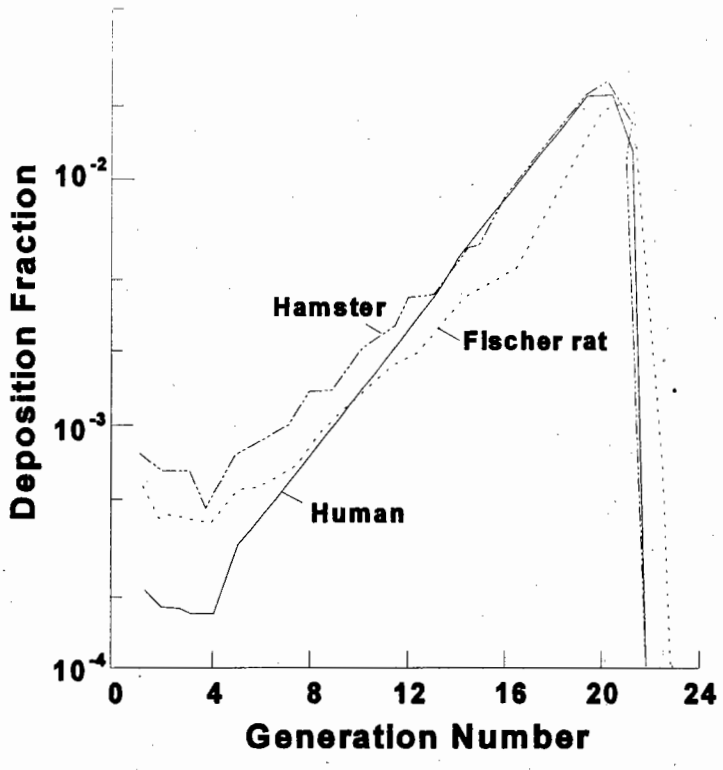

Figure 4-1. Deposition distribution patterns of inhaled diesel exhaust particles in the airways of different species.

Source: Xu and Yu (1987).

Table 4-1. Predicted doses of inhaled diesel exhaust particles per minute based on total lung volume (m), total airway surface area (M_1), or surface area in alveolar region (M_2)

Species	M (10^{-3} µg/mi/cm^3)	M_1 (10^{-6} µg/min/cm^2)	M_2 (10^{-6} µg/min/cm^2)
Hamster	3.548	3.088	2.382
Fischer rat	3.434	3.463	2.608
Human	0.249	1.237	0.775

M and M_1 = mass of particles deposited in total lung.
M_2 = mass of particles deposited in the alveolar region only.
Based on the following conditions: (1) MMAD = 0.2 µm, σ = 1.9, ϕ = 0.3, and ρ = 1.5 g/cm^3; (2) particle concentration = 1 mg/m^3; and (3) nose-breathing.

Source: Xu and Yu (1987).

volume basis (Table 4-1). This is due to larger alveolar diameters in humans and concomitantly lower surface area per unit of lung volume.

The alternative, perhaps more accurate physiologically, is to consider deposition rate relative to exposure concentration; the deposition rate will initiate particle redistribution processes (e.g., clearance mechanisms, phagocytosis) that transfer the particles to various subcompartments, including the alveolar macrophage pool, pulmonary interstitium, and lymph nodes. Over time, therefore, only small amounts of the original particle intake would be associated with the alveolar surface.

4.3. RESPIRATORY TRACT CLEARANCE RATES

4.3.1. Tracheobronchial Clearance

The dynamic relationship between deposition and clearance is responsible for determining lung burden at any point in time. Clearance of highly insoluble particles from the tracheobronchial region is mediated primarily by mucociliary transport and is a more rapid process than those operating in alveolar regions. Mucociliary transport (often referred to as the mucociliary escalator) is accomplished by the rhythmic beating of cilia that line the respiratory tract from the trachea through the terminal bronchioles. This movement propels the mucous layer containing deposited particles (or particles within alveolar macrophages [AMs]) toward the larynx. 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 interspecies and individual variability. Considerable species-dependent variability in tracheobronchial clearance has been reported, with dogs generally having faster clearance rates than guinea pigs, rats, or rabbits (Felicetti et al., 1981). The half-time ($t_{1/2}$) values for tracheobronchial clearance of relatively insoluble particles are usually on the order of hours; those for alveolar clearance may be hundreds of days in humans and dogs. The clearance of particulate matter from the tracheobronchial region is generally recognized as being biphasic or multiphasic (Raabe, 1982). Some studies have shown that particles are cleared from large, intermediate, and small airways with $t_{1/2}$ of 0.5, 2.5, and 5 h, respectively. However, reports have indicated that clearance from conducting airways is biphasic and that the long-term component for humans may take much longer for a significant fraction of particles deposited in this region and may not be complete within 24 h, as generally believed (Stahlhofen et al., 1990).

Although most of the particulate matter cleared from the tracheobronchial region will ultimately be swallowed, the contribution of this fraction relative to carcinogenic potential is unclear. With the exception of conditions of impaired bronchial clearance, the desorption $t_{1/2}$ for particle-associated organics is generally longer than the tracheobronchial clearance times,

thereby making uncertain the importance of this fraction relative to carcinogenesis in the respiratory tract (Pepelko, 1987). Gerde et al. (1991a) showed that for low-dose exposures, particle-associated PAHs were rapidly released, thereby suggesting retained particle-associated PAHs may be of lesser importance in tumorigenic responses than originally believed. The relationship between the early clearance of insoluble particles (4 μm aerodynamic diameter) from the tracheobronchial regions and their longer-term clearance from the alveolar region is illustrated in Figure 4-2.

Cuddihy and Yeh (1986) reviewed respiratory tract clearance of particles inhaled by humans. Depending on the type of particle (ferric oxide, Teflon discs, or albumin microspheres), the technique employed, and the anatomic region (midtrachea, trachea, or main bronchi), particle velocity (moved by mucociliary transport) ranged from 2.4 to 21.5 mm/min. The highest velocities were recorded 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).

Cuddihy and Yeh (1986) described salient points to be considered when estimating particle clearance velocities from tracheobronchial regions: 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. The velocities observed for inhalation studies were generally lower than those of instillation studies. Figure 4-3 illustrates a comparison of the short-term clearance of inhaled particles by human subjects and the model predictions for this clearance. However, tracheobronchial clearance via the mucociliary escalator is of limited importance for long-term retention.

Exposure of F344 rats to whole DPM at concentrations of 0.35, 3.5, or 7.0 mg/m^3 for up to 24 mo did not significantly alter tracheal mucociliary clearance of ^{98m}Tc-macroaggregated albumin instilled into the trachea (Wolff et al., 1987). The assessment of tracheal clearance was determined by measuring the amount of material retained 1 h after instillation. The authors stated that measuring retention would yield estimates of clearance efficiency comparable to measuring the velocity for transport of the markers in the trachea. The results of this study were in agreement with similar findings of unaltered tracheal mucociliary clearance in rats exposed to DPM (0.21, 1.0, or 4.4 mg/m^3) for up to 4 mo (Wolff and Gray, 1980). However, the 1980 study by Wolff and Gray, as well as an earlier study by Battigelli et al. (1966), showed that acute

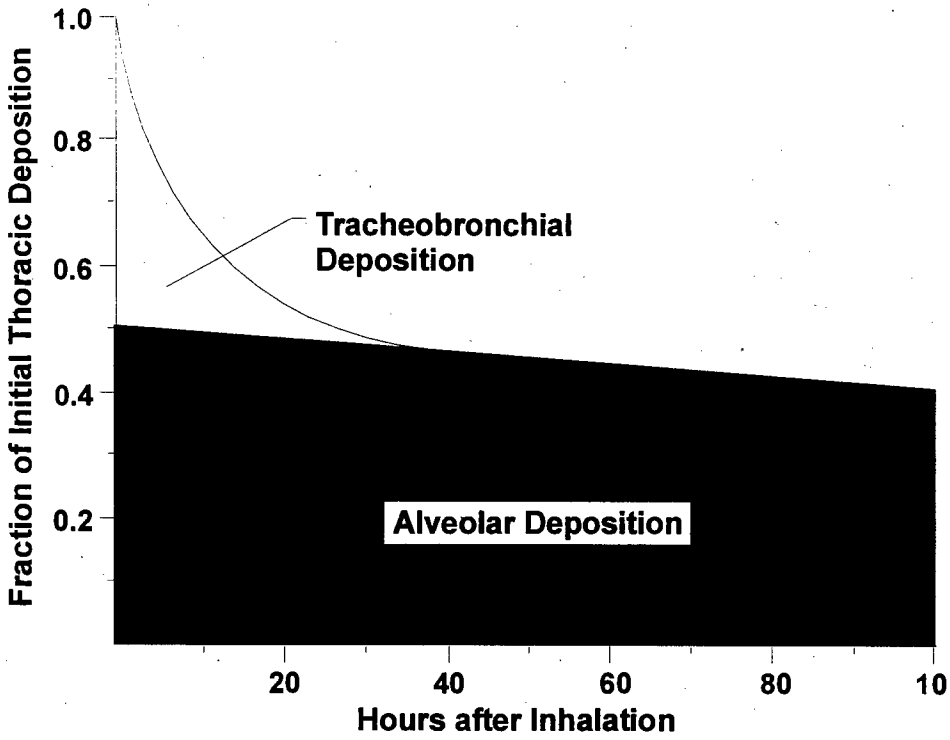

Figure 4-2. Clearance of insoluble particles deposited in tracheobronchial and alveolar regions.

Source: Chuddihy and Yeh, 1986.

exposure to high concentrations of diesel exhaust soot (1.0 and 4.4 mg/m^3 in the study by Wolff and Gray [1980] and 8 to 17 mg/m^3 in the study by Battigelli et al. [1966]) produced transient reductions in tracheal mucociliary clearance. Battigelli et al. (1966) also noted that the compromised tracheal clearance was not observed following cessation of exhaust exposure.

The fact that tracheal clearance does not appear to be significantly impaired or is impaired only transiently following exposure to high concentrations of DPM is consistent with the absence of pathological effects in the tracheobronchial region of the respiratory tract in experimental animals. However, the apparent retention of a fraction of the deposited dose in the airways is cause for some concern regarding possible carcinogenic effects in this region, especially in light of the results from simulation studies by Gerde et al. (1991b) that suggested that release of polycyclic aromatic hydrocarbons (PAHs) from particles may occur within minutes and at the site of initial deposition. Moreover, impairment of mucociliary clearance function as a result of exposure to either occupational or environmental respiratory tract toxicants

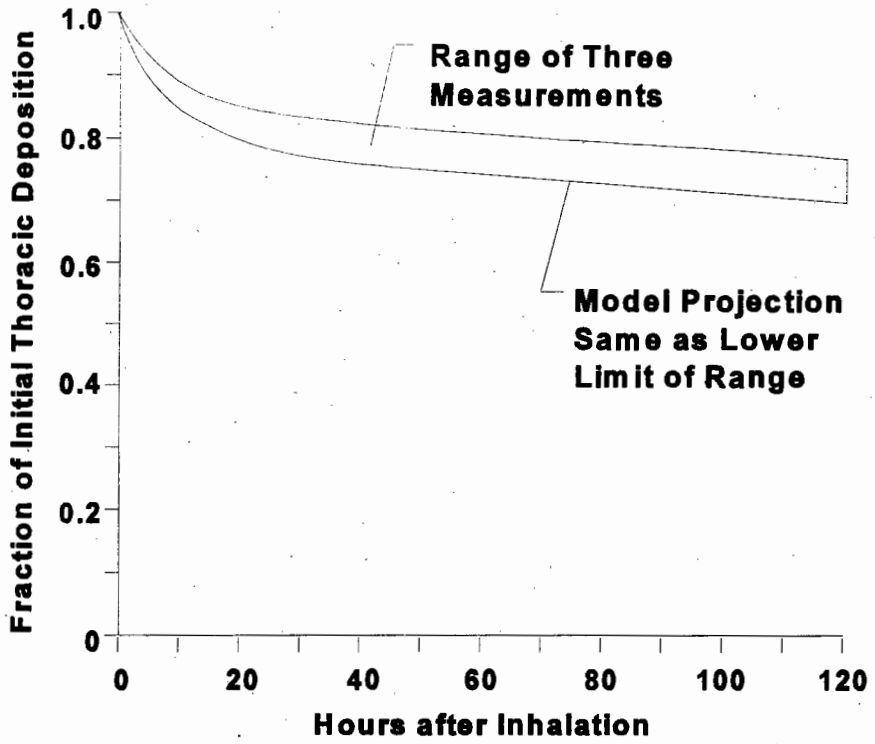

Figure 4-3. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

Source: Cuddihy and Yeh, 1986 (from Stahlhofen et al., 1980).

or to cigarette smoke will significantly enhance the retention of particles in this region. For example, Vastag et al. (1986) demonstrated that not only smokers with clinical symptoms of bronchitis but also symptom-free smokers have significantly reduced mucociliary clearance rates. Although impaired tracheobronchial clearance could conceivably have an impact on the effects of deposited DPM in the conducting airways, it does not appear to be relevant to the epigenetic mechanism likely in diesel exhaust-induced rat pulmonary tumors.

4.3.2. Clearance From the Alveolar Region

4.3.2.1. *Alveolar Clearance in Humans*

A number of investigators have reported on the alveolar clearance kinetics of human subjects. Bohning et al. (1980) examined alveolar clearance in eight humans who had inhaled <0.4 mg of ^{85}Sr-labeled polystyrene particles (3.6 ± 1.6 μm diam.). A double-exponential model best described the clearance of the particles and provided $t_{1/2}$ values of 29 ± 19 days and 298 ± 114 days for short-term and long-term phases, respectively. It was noted that of the particles deposited in the alveolar region, 75% ± 13% were cleared via the long-term phase. Alveolar

retention $t_{1/2}$ values of 330 and 420 days were reported for humans who had inhaled aluminosilicate particles (Bailey et al., 1982).

Quantitative data on clearance rates in humans having large lung burdens of particulate matter are lacking. Bohning et al. (1982) and Cohen et al. (1979), however, did provide evidence for slower clearance in smokers, and Freedman and Robinson (1988) reported slower clearance rates in individuals who had mild pneumoconiosis. Although information on particle burden and particle overload relationships in humans is much more limited than for experimental animal models, inhibition of clearance does seem to occur. Stöber et al. (1967) estimated a clearance $t_{1/2}$ of 4.9 years in coal miners with nil or slight silicosis, based on postmortem lung burdens. The lung burdens ranged from 2 to 50 mg/g of lung or more, well above the value for which sequestration is observed in the rat. Furthermore, impaired clearance resulting from smoking or exposure to other respiratory toxicants may increase the possibility of an enhanced particle accumulation effect resulting from exposure to other particle sources such as diesel exhaust.

4.3.2.2. *Alveolar Clearance in Animals*

Normal alveolar clearance rates in animals have been reported by a number of investigators. Because the rat is the species for which experimentally induced lung cancer data are available and for which most clearance data exist, it is the species most often used for assessing human risk, and reviews of alveolar clearance studies have been generally limited to this species.

Chan et al. (1981) subjected 24 male F344 rats to nose-only inhalation of DPM (6 mg/m^3) labeled with ^{131}Ba or ^{14}C for 40 to 45 min and assessed total lung deposition, retention, and elimination. Based on radiolabel inventory, the deposition efficiency in the respiratory tract was 15 to 17%. Measurement of ^{131}Ba label in the feces during the first 4 days following exposure indicated that 40% of the deposited DPM was eliminated via mucociliary clearance. Clearance of the particles from the lower respiratory tract followed a two-phase elimination process consisting of a rapid ($t_{½}$ of 1 day) elimination by mucociliary transport and a slower ($t_{½}$ of 62 days) macrophage-mediated alveolar clearance. This study provided data for normal alveolar clearance rates of DPM not affected by prolonged exposure or particle overloading.

Several studies have investigated the effects of exposure concentration on the alveolar clearance of DPM by laboratory animals.

Wolff et al. (1986, 1987) provided clearance data ($t_{½}$) and lung burden values for F344 rats exposed to diesel exhaust for 7 h/day, 5 days/week for 24 mo. Exposure concentrations of 0.35, 3.5, and 7.0 mg of soot/m^3 were employed in this whole body-inhalation exposure experiment. Intermediate (hours-days) clearance of $^{67}Ga_2O_3$ particles (30 min, nose-only

inhalation) was assessed after 6, 12, 18, and 24 mo of exposure at all of the DPM 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 throughout the respiratory tract, *A* and *B* were the magnitudes of the two components (component *A* representing the amount cleared from nasal, lung, and gastrointestinal compartments and component *B* representing intermediate clearance from the lung compartment), and τ_1 and τ_2 were the half-times for the *A* and *B* compartments, respectively. The early retention 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, whose clearance rate was faster than that of the controls. Significantly longer *B* compartment retention half-times, representing the early clearance from nonciliated passages such as alveolar ducts and alveoli, were noted after as few as 6 months of exposure to DPM at 7.0 mg/m^3 and 18 months of exposure to 3.5 mg/m^3.

Nose-only exposures to ^{134}Cs fused aluminosilicate particles (FAP) were used to assess long-term (weeks-months) clearance. Following 24-month exposure to DPM, long-term clearance of ^{134}Cs-FAP was significantly ($p<0.01$) altered in the 3.5 (cumulative exposure [C × T] of 11,760 mg·h/m^3) and 7.0 mg/m^3 (C × T = 23,520 mg·h/m^3) exposure groups ($t_{1/2}$ of 264 and 240 days, respectively) relative to the 0.35 mg/m^3 and control groups ($t_{1/2}$ of 81 and 79 days, respectively). Long-term clearance represents the slow component of particle removal from the alveoli. The decreased clearance correlated with the greater particle burden in the lungs of the 3.5 and 7.0 mg/m^3 exposure groups. Based on these findings, the cumulative exposure of 11,760 mg·h/m^3 represented a particle overload condition resulting in compromised alveolar clearance mechanisms.

Heinrich et al. (1986) exposed rats 19 h/day, 5 days/week for 2.5 years to DPM at a particle concentration of about 4 mg/m^3. This is equal to a C × T of 53,200 mg·h/m^3. The deposition in the alveolar region was estimated to equal 60 mg. The lung particle burden was sufficient to result in a particle overload condition. With respect to the organic matter adsorbed onto the particles, the authors estimated that over the 2.5-year period, 60 to 150 mg of particle-bound organic matter had been deposited and was potentially available for biological effects. This estimation was based on the analysis of the diesel exhaust used in the experiments, values for rat ventilatory functions, and estimates of deposition and clearance.

Accumulated burden of DPM in the lungs following an 18-mo, 7 h/day, 5 days/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 DPM/m^3 were sacrificed at 1 day and 1, 5, 15, 33, and 52 weeks after exposure, and DPM was extracted from lung tissue dissolved in tetramethylammonium hydroxide. Following centrifugation and washing of the supernatant, DPM content of the tissue was quantitated using spectrophotometric techniques. The analytical procedure was verified by comparing results to recovery studies using known amounts of DPM with lungs of unexposed rats. Long-term retention for the 0.15 and 0.94 mg/m^3 groups had estimated half-times of 87 ± 28 and 99 ± 8 days, respectively. The retention $t_{1/2}$ for the 4.1-mg/m^3 exposure group was 165 ± 8 days, which was significantly (p<0.0001) greater than those of the lower exposure groups. The 18-mo exposures to 0.15 or 0.96 mg/m^3 levels of DPM (C × T equivalent of 378 and 2,368 mg·h/m^3, respectively) did not affect clearance rates, whereas the exposure to the 4.1 mg/m^3 concentration (C × T = 10,332 mg·h/m^3) resulted in significant lung DPM burdens and impaired clearance.

In a subsequent study (Lee et al., 1983), a three-phase model was used to describe the clearance of DPM (7 mg/m^3 for 45 min or 2 mg/m^3 for 140 min) by F344 rats (24 per group) exposed by nose-only inhalation with no apparent particle overload in the lungs. The exposure protocols provided comparable total doses based on a ^{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 days after exposure and resulted in the derivation of a three-phase clearance of the particles. The resulting retention $t_{1/2}$ values for the three phases were 1, 6, and 80 days. The three clearance phases are taken to represent removal of tracheobronchial deposits by the mucociliary escalator, removal of particles deposited in the respiratory bronchioles, and alveolar clearance, respectively. Species variability in clearance of DPM was also demonstrated by the fact that Hartley guinea pigs exhibited negligible alveolar clearance from day 10 to day 432 following a 45-min exposure to a DPM concentration of 7 mg/m^3. Initial deposition efficiency (20 ± 2%) and short-term clearance were, however, similar to those for rats.

Lung clearance in male F344 rats preexposed to DPM at 0.25 or 6 mg/m^3 20 h/day, 7 days/week for periods lasting from 7 to 112 days was studied by Chan et al. (1984). Following this preexposure protocol, rats were subjected to 45-min nose-only exposure to ^{14}C-diesel exhaust and alveolar clearance of radiolabel was monitored for up to 1 year. Two models were proposed: a normal biphasic clearance model and a modified lung retention model that included a slow-clearing residual component to account for sequestered aggregates of macrophages. The first model described a first-order clearance for two compartments: $R(t) = Ae^{-u1t} + Be^{-u2t}$. This yielded clearance $t_{1/2}$ values of 166 and 562 days for rats preexposed to 6.0 mg/m^3 for 7 and 62 days, respectively. These values were significantly (p<0.05) greater than the retention $t_{1/2}$ of 77 ±

17 days for control rats. The same retention values for rats of the 0.25 mg/m^3 groups were 90 ± 14 and 92 ± 15 days, respectively, for 52- and 112-day exposures and were not significantly different from controls. The two-compartment model represents overall clearance of the tracer particles, even if some of the particles were sequestered in particle-laden macrophages with substantially slower clearance rates. For the second model, which excluded transport of the residual fractions in sequestered macrophage aggregates, slower clearance was observed in the group with a lung burden of 6.5 mg, and no clearance was observed in the 11.8 mg group. Clearance was shown to be dependent on the initial burden of particles and, therefore, the clearance $t_{1/2}$ would increase in higher exposure scenarios. This study emphasizes the importance of particle overloading of the lung and the ramifications on clearance of particles; the significant increases in half-times indicate an increasing impairment of the alveolar macrophage mobility and subsequent transition into an overload condition. Based on these data, a particle overload effect was demonstrated for both the high and low exposure levels (equivalent to C × T dose of 840 [transitional overload] and 7,440 mg·h/m^3).

Long-term alveolar clearance rates of particles in various laboratory animals and humans have been reviewed by Pepelko (1987). Although retention $t_{1/2}$ varies both among and within species and is also dependent on the physicochemical properties of the inhaled particles, the retention $t_{1/2}$ for humans is generally much longer (>8 mo) than the average retention $t_{1/2}$ of 60 days for rats.

4.3.2.3. *Lung Burden and Pulmonary Overload Resulting in Impaired Clearance*

Particle overload appears to be an important factor in the diesel emission-induced pulmonary carcinogenicity observed in rats. Studies described in this section provide additional data showing a particle overload effect. A study by Griffis et al. (1983) demonstrated that exposure (7 h/day, 5 days/week) of rats to DPM at concentrations of 0.15, 0.94, or 4.1 mg/m^3 for 18 mo resulted in lung burdens of 35, 220, and 1,890 μg/g of lung tissue, respectively. The alveolar clearance of those rats with the highest lung burden (1,890 μg/g of lung) was impaired, as determined by a significantly greater (p<0.0001) retention $t_{1/2}$ for DPM. This is reflected in the greater lung burden/exposure concentration ratio at the highest exposure level. Similarly, in the study by Chan et al. (1984) rats exposed for 20 h/day, 7 days/week to DPM (6 mg/m^3) for 112 days had a total lung particle burden of 11.8 mg, with no alveolar particle clearance being detected over 1 year.

Muhle et al. (1990) indicated that overloading of rat lungs occurred when lung particle burdens reached 0.5 to 1.5 mg/g of lung tissue and that clearance mechanisms were totally compromised at lung particle burdens ≥ 10 mg/g for particles with a specific density close to 1.

Pritchard (1989), utilizing data from a number of diesel exhaust exposure studies, examined alveolar clearance in rats as a function of cumulative exposure. The resulting analysis noted a significant increase in retention $t_{1/2}$ values at exposures above 10 mg/m^3·h/day and also showed that normal lung clearance mechanisms appeared to be compromised as the lung DPM burden approached 0.5 mg/g of lung.

Morrow (1988) has proposed that the condition of particle overloading in the lungs is caused by a loss in the mobility of particle-engorged AMs and that such an impediment is related to the cumulative volumetric load of particles in the AM. Morrow (1988) has further estimated that the clearance function of an AM may be completely impaired when the particle burden in the AM is of a volumetric size equivalent to about 60% of the normal volume of the AM. Morrow's hypothesis was the initial basis for the physiology-oriented multicompartmental kinetic (POCK) model derived by Stöber et al. (1989) for estimating alveolar clearance and retention of biologically insoluble, respirable particles. The model provides for physiology-oriented multicompartmental considerations and is based on the dominant role of alveolar macrophages.

A revised version of this model refines the characterization of the macrophage pool by including both the mobile and immobilized macrophages (Stöber et al., 1994). A diagram of the POCK model is shown in Figure 4-4. For characterizing these macrophage tools, the model assumes a constant maximum volume capacity of the macrophages for particle uptake and a material-dependent critical macrophage load that results in total loss of macrophage mobility. Application of the model to experimental data suggested that lung overload does not cause a dramatic increase in the total burden of the macrophage pool but results in a great increase of the particle burden of the interstitial space, a compartment that is not available for macrophage-mediated clearance. The model predictions, however, regarding the subcompartmental concentrations of particles require an experimental database at a reasonably wide range of external dose rates.

Oberdörster and co-workers (1992) assessed the alveolar clearance of smaller (3.3 μm diam.) and larger (10.3 μm diam.) polystyrene particles, the latter of which are volumetrically equivalent to about 60% of the average normal volume of a rat AM, after intratracheal instillation into the lungs of rats. Even though sizes of particles were found to be phagocytized by AM within a day after deposition and the smaller particles were cleared at a normal rate, only minimal lung clearance of the larger particles was observed over an approximately 200-day postinstillation period, thus supporting the volumetric overload hypothesis.

Animal studies have revealed that impairment of alveolar clearance can occur following chronic exposure to DPM (Griffis et al., 1983; Wolff et al., 1987; Vostal et al., 1982; Lee et al., 1983) or a variety of other diverse aerosols (Lee et al., 1986; Lee et al., 1988; Ferin and

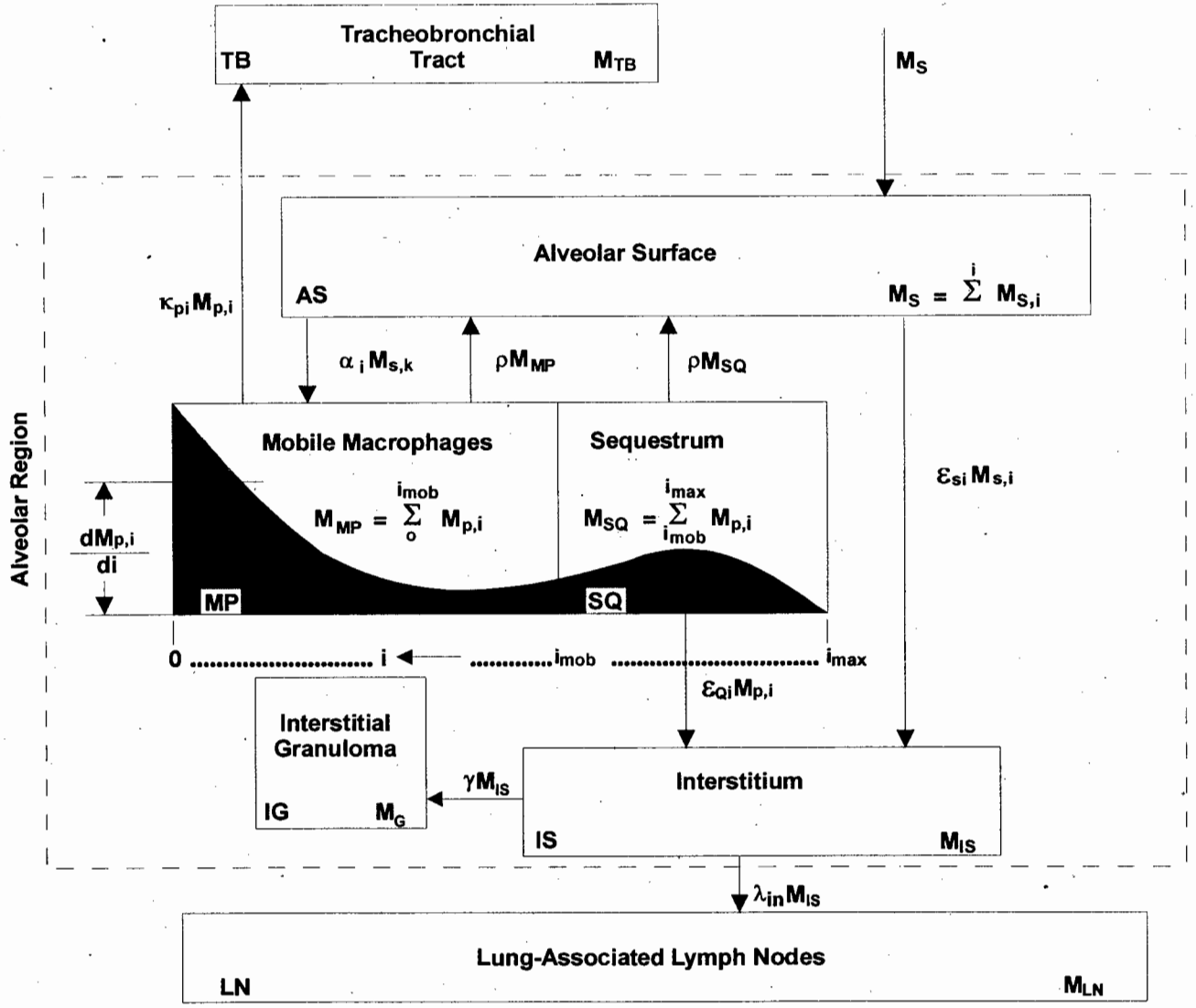

Figure 4-4. Physiology-oriented compartmental kinetics model of retention of inhaled insoluble particles in rats.

Source: Stöber et al. (1994).

Feldstein, 1978; Muhle et al. 1990). Because high lung burdens of insoluble biochemically inert particles result in diminution of normal lung clearance kinetics or in what is now called "particle overloading," this effect 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. It must be noted, however, that some types of particles may impair clearance at lower lung burdens (e.g., silica may impair clearance at much lower lung burdens than DPM). This could be due to toxic agents adsorbed to the particle surface, or in the case of silica, surface-associated organics. 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.

Although quantitative data are limited regarding lung overload associated with impaired alveolar clearance in humans, impairment of clearance mechanisms appears to occur, and at a lung burden generally in the range reported to impair clearance in rats. Stöber et al. (1967), in their study of coal miners, reported lung particle burdens of 2 to 50 mg/g lung tissue for which estimated clearance $t_{1/2}$ values were very long (4.9 years). Freedman and Robinson (1988) also reported slower alveolar clearance rates in coal miners, some of whom had a mild degree of pneumoconiosis. It must be noted, however, that no lung cancer was reported for those miners with apparent particle overload.

4.3.3. Role of AMs in the Clearance of Particulate Matter

4.3.3.1. *AM-Mediated Clearance of Particulate Matter*

Alveolar macrophages constitute an important first-line cellular defense mechanism against inhaled particles that deposit in the alveolar region of the lung. It is well established that a host of diverse materials, including DPM, are phagocytized by the AMs shortly after deposition (White and Garg, 1981; Lehnert and Morrow, 1985) and that such cell-contained particles are generally rapidly sequestered from both the extracellular fluid lining in the alveolar region and the potentially sensitive alveolar epithelial cells. In addition to this role in compartmentalizing particles from other lung constituents, AMs are 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, AMs with their particle burdens gain access and become coupled to the mucociliary escalator and are subsequently transported from the lung via the conducting airways. Although 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 (Gibb and Morrow, 1962; Ferin, 1982; Harmsen et al., 1985; Lehnert and Morrow, 1985; Powdrill et al., 1989).

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 in which each component follows simple first-order kinetics (Snipes and Clem, 1981; Snipes et al., 1988; Lee et al., 1983). Although the various compartments can be described mathematically, the actual physiologic mechanisms determining these differing clearance rates have not been well characterized.

Lehnert et al. (1988, 1989) 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 microspheres (2.13 μm diam.) to obtain information on potential AM-related mechanisms that form the underlying bases for kinetic patterns of alveolar clearance as a function of particle lung burdens. The intratracheally instilled lung burdens studied varied from 1.6×10^7 particles (about 85 μg) for the low lung burden to 2.0×10^8 particles (about 1.06 mg) for the mid-dose and 6.8×10^8 particles (about 3.6 mg) for the highest lung burden. The lungs were lavaged at various times postexposure and the numbers of spheres in each macrophage counted. Although such experiments provide information regarding the response of the lung to particulate matter, intratracheal instillation is not likely to result in the same depositional characteristics as inhalation of particles would. Therefore, it is unlikely that the response of alveolar macrophages to these different depositional characteristics will be quantitatively similar.

The $t_{1/2}$ values of both the early and later components of clearance were virtually identical following deposition of the low and medium lung burdens. For the highest lung burden, significant prolongations were found in both the early, more rapid as well as the slower component of alveolar clearance. The percentages of the particle burden associated with the earlier and later components, however, were similar to those of the lesser lung burdens. On the basis of the data, the authors concluded that translocation of AMs from alveolar spaces by way of the conducting airways is fundamentally influenced by the particle burden of the cells so translocated. In the case of particle overload that occurred at the highest lung burden, the translocation of AMs with the heaviest cellular burdens of particles (i.e., greater than about 100 microspheres per AM) was definitely compromised.

On the other hand, analysis of the disappearance of AMs with various numbers of particles indicates that they 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 AMs concurrent with the removal of particle-containing AMs via the conducting airways per se. Experimental support suggestive of potential processes for such particle redistribution comes from a variety of investigations involving AM and other endocyte cell types (Heppleston

and Young, 1974; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978).

4.3.3.2. *Translocations of Particles to Extraalveolar Macrophage Compartment Sites*

Although the phagocytosis of particles by lung-free cells and the mucociliary clearance of the cells with their particulate matter burdens represent the most prominent mechanisms that govern the fate of particles deposited in the alveolar region, other mechanisms exist that can affect both the retention characteristics of relatively insoluble particles in the lung and the lung clearance pathways for the particles. One mechanism is endocytosis of particles by Type I cells (Sorokin and Brain, 1975; Adamson and Bowden, 1978, 1981) that normally provide >90% of the cell surface 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 the particles that deposit in the lungs and the numbers of particles that are deposited. Adamson and Bowden (1981) found that with increasing loads of carbon particles (0.03 μm diam.) instilled in the lungs of mice, more free particles were observed in the alveoli within a few days. The relative abundance of particles endocytosed by Type I cells also increased with increasing lung burdens of the particles, but instillation of large particles (1.0 μm) rarely resulted in their undergoing endocytosis. A 4 mg burden of 0.1 μm diameter latex particles is equivalent to 8×10^{12} particles, whereas a 4 mg burden of 1.0 μm particles is composed of 8×10^{9} particles. Regardless, DPM with volume median diameters between 0.05 and 0.3 μm (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 DPM is endocytosed by Type I cells in vivo (White and Garg, 1981).

Unfortunately, information on the kinetics of particle endocytosis by Type I cells relative to that by AMs is scanty. Even when relatively low burdens of particulate matter are deposited in the lungs, some fraction of the particles usually appears in the regional lymph nodes (Ferin and Fieldstein, 1978; Lehnert, 1989). As will be discussed, endocytosis of particles by Type I cells is an initial, early step 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 AMs that initially sequestered them after lung deposition, some fraction of the "free" particles so released could also undergo passage from the alveolar space into Type I cells.

The endocytosis of particles by Type I cells 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 cell 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 remain in a "free" state for a poorly defined period 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 AMs, and perhaps polymorphonuclear lymphocytes (PMNs) 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. Evidence that such migration of AMs may contribute significantly to the passage of particles to the interstitial compartment and also may 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 remain in a free state in the interstitium for some time without being engulfed by interstitial macrophages. It is unknown if interstitial macrophages 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 the burden of particles with an interstitial macrophage may have on the AM's ability to migrate into the alveolar space compartment.

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 Fieldstein, 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 may reach these sites as free particles. Whatever the process, existing evidence indicates that when lung burdens of particles result in a 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).

4.3.3.3. *Potential Mechanisms for an AM Sequestration Compartment for Particles During Particle Overload*

Several factors may be involved in the particle-load-dependent retardations in the rate of particle removal from the lung and the corresponding functional appearance of an abnormally slow clearing or particle sequestration compartment. As previously mentioned, one potential site for particle sequestration is the containment of particles in the Type I cells. Information on the retention kinetics for particles in the Type I cells is not 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 cell population during lung overloading.

Another anatomical region in the lung that may be a slow clearing site is the interstitial compartment. Little is known about either the kinetics of removal of free particles or particle-containing macrophages from the 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 the 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 DPM that Type I cells, the interstitium, the lymphatic channels, and pulmonary lymphoid tissues are sites that could represent subcompartments of a more generalized slow clearing compartment.

Although these sites must be considered to be potential contributors to the increased retention of particles during particle overload, a disturbance in particle-associated AM-mediated clearance is undoubtedly the predominant cause, inasmuch as the AMs are the primary reservoirs of deposited particles. The factors responsible for a failure of AMs to translocate from the alveolar space compartment in lungs with high particulate matter burdens remain uncertain, although a hypothesis concerning the process has been offered involving volumetric AM burden (Morrow, 1988).

Other processes also may be involved in preventing particle-laden AMs from leaving the alveolar compartment under conditions of particle overload in the lung. Clusters or aggregates of particle-laden AMs in the alveoli are typically found in the lungs of laboratory animals that have received large lung burdens of a variety of types of particles (Lee et al., 1985), including DPM (White and Garg, 1981; McClellan et al., 1982). The aggregation of AMs may explain, in part, the reduced clearance of particle-laden AM during particle overload. The definitive mechanism(s) responsible for this clustering of AMs has not been elucidated to date. Whatever

the underlying mechanism(s) for the AM agglutinating response, it is noteworthy that AMs 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 AMs 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 directly caused by their abundant accumulation as a result of immobilization by large particle loads. Furthermore, even though overloaded macrophages may redistribute particle burden to other AMs, clearance may remain inhibited (Lehnert, 1988). This may, in part, be due to attractants from the overloaded AMs causing aggregation of those that are not carrying a particle burden.

4.3.3.4. *Physiologically Based Models for Pulmonary Retention and Clearance of Insoluble Particles*

Currently available data for long-term inhalation exposures to insoluble particles (e.g., TiO_2, carbon black, and DPM) show that pulmonary retention and clearance of these particles are not adequately described by simple first-order kinetics and a single compartment representing the alveolar macrophage particle burden. Several investigators have developed models for deposition, transport, and clearance of insoluble particulate matter in the lungs. All of these models identify various compartments and associated transport rates, but empirically derived data are not available to validate many of the assumptions made in these models.

A two-compartment model was developed by Smith (1985) that includes alveolar and interstitial compartments. For uptake and clearance of particles by alveolar surface macrophages and interstitial encapsulation of particles (i.e., quartz dust), available experimental data show that the rate-controlling functions followed Michaelis-Menton type kinetics, while other processes affecting particle transfer are assumed to be linear. Although this model provides rate constants as functions that vary depending on the conditions within the various compartments, most of the described functions could not be validated with experimental data.

Strom et al. (1988) developed a multicompartmental model for particle retention that includes the following compartments: (1) tracheobronchial tree, (2) free particulate on the alveolar surface, (3) mobile phagocytic alveolar macrophages, (4) sequestered particle-laden alveolar macrophages, (5) regional lymph nodes, and (6) gastrointestinal tract. The model is based on mass-dependent clearance (the rate coefficients reflect this relationship), which will dictate sequestration of particles and their eventual transfer to the lymph nodes.

Yu et al. (1989) considered a three-compartment model that includes a macrophage compartment containing all of the phagocytized particles, an interstitial compartment, and a lymph node compartment. Analyzing the clearance of insoluble particles from the rat lung in

terms of alveolar macrophage mobility kinetics indicated the reduced clearance in overload conditions to be a function of reduced macrophage mobility (due to volumetric increase in cell size from phagocytized particles) and an increased deposition of particles in the interstitial space.

The most recent version of the physiology-oriented multicompartmental kinetics model derived by Stöber and co-workers (1994) (see Section 4.3.2.3., Figure 4-4) addresses alveolar clearance and retention of inhaled insoluble particles more rigorously by incorporating five subcompartments representing alveolar particle load and the respective kinetics associated with the transfer of particle load among these compartments. This model also emphasizes the importance of interstitial burden in the particle overload phenomenon and indicates that particle overload is a function of a massive increase in particle burden of the interstitial space rather than total burden of the macrophage pool. The relevance of the increased particle burden in the interstitial space lies with the fact that this compartmental burden is not available for macrophage-mediated clearance and, therefore, persists even after cessation of exposure. Although the model predictions are tenable, experimental data are not currently available to validate the proposed compartmental burdens or the transfer rates associated with these compartments. Stöber and co-workers noted that the POCK model should not be generalized beyond its design and cannot readily be used for interspecies extrapolation.

4.4. BIOAVAILABILITY OF ORGANIC CONSTITUENTS PRESENT ON DIESEL EXHAUST PARTICLES

Because it has been shown that DPM extract is not only mutagenic but also contains known carcinogens, the organic fraction was originally considered to be the primary source of carcinogenicity in animal studies. Evidence presented in more recent studies, however, indicates that the insoluble carbon core of the particle may fully explain the pathogenic and carcinogenic processes observed in the rat inhalation studies. (See Chapter 10 for a discussion of this issue.) Although the organic constituents appear to be of limited importance in rats exposed at high concentrations, their contributory potential in other species cannot be summarily discounted.

A key factor in determining a possible contributory effect is the bioavailability of particle-adsorbed components and whether or not the bioavailable fraction represents an effective dose. An initial step, and possibly the rate-limiting step, in the bioavailability of carcinogenic organics present on diesel particles is their dissociation from the particle surface (Vostal, 1983). This section will therefore focus on studies relating to the dissociation of these organics from the particles. However, the critical uncertainty in these study results is whether the organics added to particles by surface adsorption will exhibit desorption characteristics similar to organics

attached to the carbon core during pyrolytic condensation. Data are not available that provide a definitive answer to this question.

4.4.1. Laboratory Animal Studies

Several studies reported on the disposition of particle-adsorbed organics following administration to various rodent species. Most of the experiments used intratracheal instillation, although some exposed the animals by nose-only inhalation.

Sun et al. (1984) compared the disposition of diesel particle-adsorbed benzo[a]pyrene (B[a]P) (0.1% by weight) and pure B[a]P following nose-only inhalation by F344 rats. Long-term retention (percentage retained after 7 days) of particle-adsorbed ^{3}H-B[a]P was approximately 230-fold greater than that for pure ^{3}H-B[a]P. Alveolar clearance of particle-associated ^{3}H was biphasic, with a long-term $t_{1/2}$ of 18 days, the latter representing clearance of 59% of the initially deposited radiolabel. Clearance of pure B[a]P aerosol was >99% within 2 h and was apparently the result of alveolar and tracheobronchial epithelial absorption into the blood, rather than the result of mucociliary clearance and subsequent ingestion (Sun et al., 1982). The data therefore indicate that adsorption to the carbonaceous diesel particle prolongs retention of the organic components.

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 DPM, following 1-h nose-only inhalation by male F344 rats. Concentrations of ^{14}C-NP ranged from 0.05 to 1.1 mg/m^3 of air, and DPM concentrations, where used, ranged from 3.7 to 6.1 mg/m^3 of air. The results indicated that long-term lung retention of ^{14}C-NP adsorbed onto DPM was 80-fold greater ($t_{1/2}$ = 36 days) than that for pure ^{14}C-NP, demonstrating again that adsorption onto the diesel particles prolongs the release of the PAHs.

Residence time is also prolonged when organics are adsorbed to other types of particles. For example, Creasia et al. (1976) found that when crystalline B[a]P was instilled into the lungs of mice, it was removed from the respiratory tract with a $t_{1/2}$ of ~1.5 h, but when the B[a]P was adsorbed to 0.5 to 1.0 μm carbon particles, its $t_{1/2}$ in the respiratory tract increased to ~36 h. Hence, the adsorption of B[a]P to the carbon particles increased the lung retention of the B[a]P more than 20-fold. Similar results have also been obtained with B[a]P adsorbed to other particle types, including insoluble Ga_2O_3 (Sun et al., 1982) and insoluble ferric oxide (Saffiotti et al., 1964). Consistent with a gradual elution of B[a]P in AMs, Creasia and co-workers (1976) 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 ~7 days.

Ball and King (1985) studied the disposition and metabolism of ^{14}C-labeled 1-NP (>99.9% purity) coated onto DPM. A single dose of ^{14}C-NP (380 μg/g particle) was intratracheally administered (in 0.2-mL buffered saline) at a particle dose of 5 mg per rat. Another group of rats (number not specified) received the labeled ^{14}C-NP in 0.5 mL of buffered saline 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, >50% of the ^{14}C was excreted within the first 24 h; 20% to 30% of this appeared in the urine, and 40% to 60% was excreted in the feces. The ^{14}C excretion pattern for the intratracheally instilled compound was nearly identical to that of the orally administered compound. For animals receiving intratracheally instilled compound, 16% to 38% of the unexcreted dose was in the gastrointestinal tract and 5% to 8% remained in the lungs. Traces of radiolabel were detected in the trachea and esophagus. Five percent to 12% of the radiolabel in the lung co-purified with the protein fraction, indicating protein binding of the 1-NP-derived ^{14}C. However, the corresponding DNA fraction contained no ^{14}C above background levels. The similar excretion kinetics and metabolic profiles for these various routes of administration indicate that 1-NP becomes bioavailable both in the lungs and the gastrointestinal tract.

Bevan and Ruggio (1991) assessed the bioavailability of B[a]P adsorbed to DPM from a 5.7-L Oldsmobile engine. In this study, exhaust particles were supplemented with exogenous ^{3}H-B[a]P to provide 2.62 μg B[a]P/g of exhaust particles. Distribution of the radioactivity was assessed at 1, 6, 24, or 72 h after intratracheal instillation of these particles into Sprague-Dawley rats (1 mg of DPM suspended in 0.3 mL of 0.15 M NaCl). At 24 h after administration, 68.5% of the radiolabel remained in the lungs. This is approximately a 3.5-fold greater proportion than that reported by Sun et al. (1984), the difference being attributed to slower pulmonary absorption, less mucociliary transport in intratracheally instilled animals, and differences in administered dose. At 3 days following administration, over 50% of the radioactivity remained in the lungs, nearly 30% had been excreted into the feces, and the remainder was distributed throughout the body. Experiments using rats with cannulated bile ducts showed that approximately 10% of the administered radioactivity appeared in the bile over a 10-h period and that less than 5% of the radioactivity entered the feces via mucociliary transport. The in vitro elution of B[a]P into dimyristoylphosphatidylcholine (DMPC) vesicles was similar for ^{3}H-B[a]P-supplemented DPM and for native DPM (no additional B[a]P added), thereby indicating that the estimation of in vivo bioavailability of B[a]P from the ^{3}H-B[a]P-supplemented DPM was reasonably accurate.

Results of these studies using surface-bound organics showed that the retention of particle-adsorbed organics is prolonged relative to the organic chemicals alone. The studies also indicated that some portion of the adsorbed organics become bioavailable but provided no information regarding the effective dose of these organics or whether experiments using organics adsorbed by pyrolytic condensation would exhibit similar characteristics.

4.4.2. Extraction of Diesel Particle-Associated Organics in Biological Fluids

For mutagenicity testing or biochemical analysis, DPM is usually extracted with organic solvents such as dichloromethane. The efficiency of extraction may be much different, however, from that of the fluids surrounding the particles in the in vivo state. A number of studies have therefore been conducted in which attempts were made to extract DPM with serum or lung lavage fluid. The efficiency of extraction was usually estimated by performing mutagenicity tests on the extracts.

The utility of evaluating extraction by lung fluid or serum may be somewhat limited because particles deposited in the alveoli are normally rapidly ingested by AMs. However, as large lung burdens of DPM are attained, such as during chronic high-concentration exposures to DPM, AMs that become heavily laden may reach their phagocytic capacity, thereby reducing their phagocytic ability. Under these conditions, an increasing fraction of deposited particles could escape the phagocytic mechanism and be relatively more available over time in the extracellular lung fluid before (1) their removal from the lung by extramacrophagic clearance via the tracheobronchial route, (2) their subsequent engulfment by newly recruited phagocytes, and (3) their engulfment by Type I cells. Even under such conditions, a relatively large mass of the particles will be within AM phagolysosomes, where low pH and enzyme activity would be expected to act on the particles and adsorbed organics.

Particles from a 5.7-L engine operated at idle and from a 2.1-L engine operating on a cycle of varying speed and load were incubated in lavage fluid, serum, saline, albumin, dipalmitoyl lecithin, or dichloromethane (Brooks et al., 1981). The efficiency of extraction by biological fluids was only 3% to 10% that of dichloromethane, based on mutagenicity testing, and did not increase with incubation time up to 120 h. Similar findings were reported by King et al. (1981). In this study, lung lavage fluid and lung cytosol fluid extracts of DPM 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.

Siak et al. (1980) assessed the mutagenicity of material extracted from DPM 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 DPM. These investigators concluded that the mutagens in DPM would not be readily available in vivo. This conclusion lacks definitive proof because extracellular lung fluid is a complex mixture of constituents that undoubtedly have a broad range of 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 extracellular lung fluid, to extract mutagenic activity from DPM clearly do not reflect the in vivo condition.

Creasia et al. (1976) reported that when B[a]P was adsorbed onto carbon particles larger than would be expected to be easily phagocytized by AMs (15 to 30 μm), the rates of elimination of the B[a]P and the particles from the lung were virtually identical. The data thus indicate little extraction from the particles not phagocytized by AM but only surrounded by epithelial lining fluid.

In summary, because lung fluids appear to be relatively ineffective in the extraction of particle-adsorbed organics and relatively few particles escape phagocytosis, free particles are likely to contribute very little to the acute bioavailability of adsorbed organics. However, during a particle-overload condition, as occurred in the chronic inhalation studies, it can be hypothesized that an increased fraction of the deposited DPM will not be phagocytized and that their adsorbed organics could be released over a long period with a clearance rate that may be equivalent to that of the particles themselves. In this case, the organics are bioavailable for even longer periods than those from phagocytized particles, from which organics in turn are retained for longer periods than nonadsorbed organics. Data are not available, however, to validate this hypothesis, nor is it known that such extractions would result in effective doses. It is unknown if AMs in particle-overload conditions retain the same capacity to dissolve organics or whether this process is slowed as well, which would also increase the time over which carcinogenic organics are released and available in the overloaded lung. Data of Creasia et al. (1976) showing increased retention of organics adsorbed to large particles could be consistent with this possibility because 15 μm particles may still be phagocytized by AM (Snipes and Clem, 1981; Oberdörster et al., 1991).

4.4.3. Extraction of Diesel Particle-Associated Organics by Alveolar Lung Cells and Other Cell Types

Another more likely mechanism by which organic carcinogens (e.g., PAHs) may be extracted from DPM in the lung is either particle dissolution or extraction of organics from the particle surface within the phagolysosomes of AMs. This mechanism presupposes that the particles are internalized by these phagocytes. Specific details about the physicochemical conditions of the intraphagolysosomal environment, where particle dissolution in AMs 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 (some metals) by macrophages (Marafante et al., 1987; Lundborg et al., 1984), but few studies provide quantitative information concerning how organic constituents of diesel particles (e.g., B[a]P) may be extracted in the phagolysosomes (Bond et al., 1983). Whatever the mechanism, the end result is a prolonged exposure of the respiratory epithelium to the gradual release of carcinogenic agents.

Quantitative data on how readily carcinogenic organics may be extracted from DPM in the human lung are not available. As shown by Creasia et al. (1976), B[a]P adsorbed onto 15 to 30 μm carbon particles is removed from the mouse lung at the same rate as the particles. However, B[a]P adsorbed onto 0.5 to 1.0 μm carbon particles was eliminated approximately four times faster than the clearance of the carbon particles. 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 DPM over a period consistent with alveolar phase clearance was about 18 days. This latter value is similar to the $t_{1/2}$ for the removal of particles without B[a]P from the rat's lung during the early, more rapid component of alveolar phase clearance (Snipes et al., 1988; Snipes and Clem, 1981; Ferin, 1982; Lehnert et al., 1989; Lehnert, 1989). These findings may suggest that the extraction of organic components from carrier particles by AMs may differ among species, although differences in the lung burdens administered in the investigations mentioned here may have influenced the outcomes of the studies.

It should be pointed out that studies designed to examine the extraction of organics by AMs 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 DPM with in vivo kinetics that differ more or less from those of B[a]P. Thus, existing dosimetry models that incorporate desorption of B[a]P from DPM as a representative organic constituent (Yu and Yoon, 1988) may

not accurately reflect the actual bioavailability of other procarcinogenic agents on DPM. As discussed in the next section, however, any error in this respect is likely to be minor.

4.4.4. Bioavailability of Adsorbed Compounds as a Function of Particle Clearance Rates and Extraction Rates of Adsorbed Compounds

The bioavailability of toxic organic compounds adsorbed to particles can be influenced by a variety of factors. Although the agent may be active while present on the particle, most particles are taken up by AMs, a cell type not generally considered to be a target site. To reach the target site, the agent must first elute from the particle surface. Although elution can be considered a necessary step, it may not always be sufficient. The agent must then diffuse out of the AM into the extracellular fluid and be absorbed by a target cell (e.g., a Type I cell). In analyzing phagolysosomal dissolution of various ions from particles 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 DPM 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 DPM. Inability of these constituents to penetrate target cells or to diffuse into the bloodstream is another possible factor limiting bioavailability to lung target cells. Nevertheless, until further research demonstrates otherwise, it is assumed that the rate-limiting factor in the bioavailability of particle-bound organics is the desorption rate from the particle surface.

The long-term clearance $t_{1/2}$ of diesel particles from the lungs of rats in the non-overloaded state was shown to range from about 2 to 3 mo (Chan et al., 1981; Chan et al., 1984; Griffis et al., 1983; Lee et al., 1983). Clearance rate data for DPM in humans are not available. For other types of insoluble particles such as polystyrene, however, $t_{1/2}$ values are close to 1 year (Bohning et al., 1982). The clearance $t_{1/2}$ values for B[a]P and 1-NP from the diesel particle surface, on the other hand, were reported to be only about 18 and 36 days, respectively (Sun et al., 1984; Bond et al., 1986). The lower $t_{1/2}$ values for clearance of the organics compared with particles themselves indicate that most of the organics are being eluted prior to particle clearance, especially in humans.

For humans, assuming an elution $t_{1/2}$ on the order of 2 to 4 weeks, well over 90% of the organics should desorb from the particles. Even in rats, with more rapid particle clearance rates, most of the organics can be expected to be eluted. Whether particle overload in the lung results in a change in elution rates of the organics is not known. If lung burden of particulate matter is the proper dosimetric factor for induction of pathology or carcinogenesis, target organ dose

would be predicted to increase more rapidly than exposure concentration under lung overload conditions.

Gerde et al. (1991a,b) described models simulating the effect of particle aggregation and PAH content on the rate of PAH release in the lung. The investigators used three models, one of which simulated a low-dose situation where only the adsorbed layer of PAH is released from the carrier particle and two of which simulated desorption of PAHs using high PAH levels (with and without an inert carrier dust). Based on the theoretical results obtained, particle retention would be of lesser importance for low-dose situations in which particle-associated PAHs would be rapidly released at the site of particle deposition and not necessarily at the site of particle retention. Frequent low-level exposure, therefore, may result in sustained exposure of target cells and subsequently greater likelihood of tumor formation at the site of initial deposition. For the high-dose situations, as represented by instillation experiments in animals, critical doses to cells are likely to occur at the site of retention because slow release from the particles may increase the dose of metabolites (and increase the risk of tumor formation) before the parent PAH compound can be cleared from the lungs. Generally, the models suggested that the local disposition of PAHs would be more dependent on the behavior of dissolved PAHs in the tissues after their release from the carrier particles than by interactions between the PAHs and the carrier particles. The model predictions were consistent with findings from laboratory animal studies that showed longer PAH retention with higher exposures and longer retention for instillation administration versus inhalation exposure.

Studies by Gerde et al. (1993a,b,c) using beagle dogs provided additional data regarding the dosimetry of inhaled PAH supportive of the previously discussed models. In the Gerde et al. (1993a) study, the dogs were exposed to an aerosolized bolus of PAH crystals (phenanthrene or B[a]P) in a single breath. PAH clearance was measured by monitoring PAH levels in the systemic circulation. Clearance from the alveolar region was dependent on lipophilicity of the PAH; clearance of highly lipophilic PAHs (i.e., B[a]P) was limited by diffusion of the chemical through the alveolar septa, while clearance of moderately lipophilic PAHs (i.e., phenanthrene) was limited by rate of perfusion of the blood. Therefore, bronchi, with their thicker epithelia, would be at greater risk than alveoli for PAH-induced toxicity at the portal of entry. In the Gerde et al. (1993b) study, small volumes of saline containing either dissolved B[a]P or phenanthrene or a suspension of particulate solvent green or macroaggregated albumin (MAA) were instilled into the mucous lining layer of a primary bronchus or distal tracheas. The highly lipophilic B[a]P was cleared via the mucociliary escalator, some being cleared very rapidly (>90 mm/min). The portion of B[a]P that penetrated the bronchial epithelium exhibited a half-time in the range of 1.4 h, indicating a diffusion-limited uptake of B[a]P by the airways. Although mucociliary

clearance is rapid for most of the lipophilic toxicants, the long retention time for the portion that penetrates the epithelium is sufficient for substantial metabolism to occur, resulting in a potential for local toxicity. Gerde et al. (1993c) used the data from the previously described studies to validate models of alveolar clearance (Gerde et al., 1991b), mucous lining penetration (Gerde and Scholander, 1987), and bronchial wall penetration (Gerde et al., 1991b). The analysis provided a reasonable validation of the transport models for these structural regions of the lung. Specifically, alveolar clearance and mucociliary clearance are primarily via molecular diffusion, whereas clearance from bronchial walls involves diffusion, metabolism of a portion of the PAH load, and endocytosis. Such findings suggest that the bronchial epithelium may be especially vulnerable to toxicity induced by diffusion-limited lipophilic substances.

4.5. CONSIDERATIONS FOR DOSIMETRY MODELING

Although more than one approach is possible in the development of dosimetry models for inhaled DPM, several dosimetry parameters are common to any approach. These include ventilatory rates and volumes, tracheobronchial and alveolar surface area, tracheobronchial and alveolar deposition efficiency, and tracheobronchial and alveolar clearance rates. If the particle-adsorbed organics are considered relevant to the carcinogenic response of the lung, elution $t_{1/2}$ values of these chemicals should be included in the model. The dosimetry models must consider not only species differences but also the effects of extrapolating from high exposure concentrations, which result in an inhibition of particle clearance from the lungs caused by overload. Some models include additional parameters such as transport to lung-associated lymph nodes and clearance of the interstitial compartment, but experimental data are not available to validate many of the assumptions used in these models.

An important consideration is ventilation rate. Numerous estimates of alveolar ventilation rates are available for both humans and rats. Nevertheless, such estimates are still a source of considerable potential error because they are usually resting values. Actual respiration can vary widely with activity or with exposure conditions. Human activity levels are also highly variable. Although it may be necessary to use a single mean value in a model, it would be useful to include risk estimates for individuals having much greater daily ventilation exchange rates resulting from participation in endurance sports or performance of heavy labor.

Another variable not discussed previously is the adjustment of dose based on metabolic rate. It has been EPA policy to consider that effective dose varies with metabolic rate. Arguments for and against this presumption are beyond the scope of this document. The primary consideration here is the degree of adjustment. EPA has traditionally adjusted for species differences in metabolic rate based on the 2/3 power of body weight as a surrogate for body

surface area. This factor is being reappraised within the Agency, and a preliminary proposal to alter this adjustment to the 3/4 power has been made.

A major consideration in the development of dosimetry models is a judgment concerning which fraction of exhaust is responsible for inducing lung cancer. As is discussed in Chapter 10, two approaches are used for quantitative assessment based on animal data. In the first, cancer is assumed to be induced by the organic constituents present on the particle surface, primarily PAHs and nitropyrenes. As discussed herein, in an appropriate model, the effective dose will correlate closely with the deposited dose, with only minor corrections for lung overloading. In the second approach, it is assumed that retained particle burden in the lung fully accounts for the induced lung tumors. A more specific dose parameter for this second approach may be the surface area of the retained particles, as discussed in Chapter 10. In studies with fine and ultrafine TiO_2 particles, Oberdörster et al. (1994) reported that the ultrafine particles resulted in prolonged pulmonary retention, increased translocation to and persistence in the interstitium, increased Type II cell proliferation, presence of fibrotic foci, and impairment of alveolar macrophage function in rats exposed for 12 weeks. Overall, there was a notable correlation between particle surface area and effects. In this case, modeling must account for slowing of clearance during lung overload as well as large differences in normal clearance rates between rats and humans. For this approach, if exposures are at overload levels, low-dose extrapolation may result in a risk estimate considerably different from one based on target organ dose of organics. A third approach could be proposed in which both exhaust components may be operating simultaneously, with the PAHs initiating the carcinogenic process and the particles promoting the process by inducing cell proliferation.

4.6. SUMMARY

Because only very limited evidence exists for diesel exhaust-induced tumors at non-pulmonary sites or for tumor induction by the gaseous fraction alone, dosimetry considerations were limited to either whole exhaust or particulate matter deposited in the lungs. Dosimetric considerations were further limited to the alveolar region of the respiratory tract for several reasons. First of all, most deposited particulate matter is transported by mucociliary transport from the conducting airways in less than 1 day, and exposure to diesel exhaust does not appear to significantly inhibit this process. However, as previously discussed, there may also be a long-phase retention, especially if tracheobronchial clearance is impaired. In general, the rapid clearance in the conducting airways reduces the time for extraction of organics from the particle surface (although there is evidence for some relatively rapid removal of organics), and some

AMs in the mucociliary escalator may contain particles desorbed of organics. Finally, most of the pathologic and carcinogenic effects occur at or distal to the terminal bronchioles in the rat.

Clearance of DPM from the alveolar region varied from about 2 mo in rats to an estimate of 1 year in humans. Under high-exposure regimes, lung overload occurred in rats, leading to slower or near cessation of clearance, thereby increasing lung burdens even further. In addition, with large lung burdens, uptake of particles by Type I cells, passage into the interstitium, and transport to lung-associated lymph nodes were increased. Factors considered to be involved in clearance inhibition included loss of AM mobility with large particle loads and a tendency for AMs to aggregate and become immobilized.

Most biological fluids tested, including lung lavage fluid and serum, were relatively ineffective in the extraction of organic agents adsorbed to the diesel particle surface. Particles deposited in the alveolar region, however, are rapidly phagocytized by AMs, which are more effective in this regard. Although actual elution $t_{1/2}$ values of organics from phagocytosed particles were difficult to obtain, they were generally less than those for the particles themselves, indicating that most organics are released even without inhibition of particle clearance. The gradual elution also prolonged the residence time of organics in the lungs compared with pure organic agents such as B[a]P, possibly avoiding overloading of biological activation systems and thus increasing their effectiveness.

In the development of a dosimetry model to allow both low-dose extrapolation and extrapolation of DPM bioassay data from laboratory animals to humans, several parameters must be accounted for. These include deposition efficiency, particle clearance rates, desorption rates of organics from the particle surface (if other than a particle effect is being considered), lung surface area, and particle surface area. The respiratory rates and volumes are highly variable in both laboratory animals and humans and are also determinants of deposition efficiency. Animal estimates are often based on published values collected under resting conditions. Respiration, however, may be inhibited by the irritant gases present in diesel exhaust, although less so at low dilution ratios. On the other hand, respiration may be either greater or less than estimated resting values, depending on whether exposures were carried out at night when the animals are likely to be awake and active or during the day when they are more likely to be asleep. Human respiratory exchange rates are also quite variable, with the physically active segment of the population at potentially greater risk because of higher doses resulting from higher respiration rates.

Adjustment for particle clearance rate is necessary for two reasons. First of all, many of the laboratory animal experiments were conducted under exposure regimes resulting in an inhibition of clearance caused by an accompanying lung burden overload. If lung burden of

particulate matter is considered to be the proper dosimetric variable, then the disproportionately large lung burdens at high levels of exposure must be adjusted for. Second, even under low-exposure regimes, clearance is slower in humans than in rats. If the correct dosimetric variable, on the other hand, is particle-free organic matter, a smaller adjustment for variations in particle clearance rates is required because most of the organics are likely to be eluted from the particles deposited in the alveolar region, even at normal clearance rates. Nevertheless, some adjustment is still necessary, because the organics are seldom all eluted.

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5. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

The objective of this chapter is to evaluate the noncarcinogenic 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.

5.1. HEALTH EFFECTS OF WHOLE EXHAUST

5.1.1. Human Data

5.1.1.1. *Short-Term Exposures*

In a controlled human study, Rudell et al. (1990) exposed eight healthy, nonsmoking subjects for 1 h to diluted diesel exhaust. The exposure atmosphere corresponded to about 100 $\mu g/m^3$ of particles, 3.7 ppm NO, 1.6 ppm NO_2, and 0.5 mg/m^3 formaldehyde (WHO, 1994). Bronchoalveolar lavage was performed prior to exposure and after exposure. Neutrophils were significantly increased after exposure, and the phagocytosis of opsonized yeast cells in vitro by alveolar macrophages was reduced after exposure. Lymphocytes were unchanged. This study provides an intriguing glimpse of the effect of low-level diesel exposure in humans, but only one exposure level was used, the number of subjects was low, and a limited range of endpoints was reported, so the data are inadequate to generalize about the human response. To date, no well-controlled chamber study has been conducted using methodologies for assessing subtle lung inflammatory reactions.

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

Kahn et al. (1988) reported the occurrence of 13 cases of acute overexposure to diesel exhaust among Utah and Colorado coal miners. Twelve miners had symptoms of mucous

membrane irritation, headache, and lightheadedness. Eight individuals reported nausea; four reported a sensation of unreality; four reported heartburn; three reported weakness, numbness, and tingling in their extremities; three reported vomiting; two reported chest tightness; and two others reported wheezing. Each miner lost time from work because of these symptoms, which resolved within 24 to 48 h. No air monitoring data were presented; poor work practices were described as the predisposing conditions for overexposure.

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

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

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

5.1.1.1.1. ***Diesel exhaust odor.*** The odor of diesel exhaust is considered by most people to be objectionable; at high intensities, it may produce sufficient physiological and psychological effects to warrant concern about public health. The intensity of the odor of diesel exhaust is an exponential function of its concentration such that a tenfold change in the concentration will alter the intensity of the odor by one unit. Two human panel rating scales have been used to measure diesel exhaust odor intensity. In the first (Turk, 1967), combinations of odorous materials were selected to simulate diesel exhaust odor; a set of 12 mixtures, each having twice the concentration of that of the previous mixture, is the basis of the diesel odor intensity scale (D-scale). The second method is the TIA (total intensity of aroma) scale based on seven steps, ranging from 0 to 3, with 0 being undetectable, 1/2 very slight, and 1 slight and increasing in

one-half units up to 3, strong (Odor Panel of the CRC-APRAC Program Group on Composition of Diesel Exhaust, 1979; Levins, 1981).

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

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

5.1.1.1.2. ***Pulmonary and respiratory effects***. Battigelli (1965) exposed 13 volunteers to three dilutions of diesel exhaust obtained from a one-cylinder, four-cycle, 7-hp diesel engine (fuel type unspecified) and found that 15-min to 1-h exposures had no significant effects on pulmonary resistance. Pulmonary resistance was measured by plethysmography utilizing the simultaneous recording of esophageal pressure and airflow determined by electrical differentiation of the volume signal from a spirometer. The units of concentration of the constituents in the three diluted exhausts were 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. Particle concentrations were not reported.

A number of studies have evaluated changes in pulmonary function occurring over a workshift in workers occupationally exposed to diesel exhaust (specific time period not always reported but assumed to be 8 h). In a study of coal miners, Reger 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-exposed miners, an amount not substantially different from reductions seen in non-diesel-exposed miners (0.02 and 0.04 L, respectively). Decrements in peak expiratory flow rates were similar between diesel and non-diesel exhaust-exposed miners. Miners with a history of smoking had an increased number of decrements over the shift than nonsmokers did. Although the monitoring data were not reported, the authors stated that there was no relationship between the low concentrations of measured respirable dust or NO_2 (personal samplers) when compared with shift changes for any lung function parameter measured for the diesel-exposed miners. This

study is limited because results were preliminary (abstract) and there was incomplete information on the control subjects.

Ames et al. (1982) compared the pulmonary function of 60 coal miners exposed to diesel exhaust with that of a control group of 90 coal miners not exposed to diesel exhaust for evidence of acute respiratory effects associated with exposure to diesel exhaust. Changes over the workshift in FVC, FEV_1, and forced expiratory flow rate at 50% FVC (FEF_{50}) were the indices for acute respiratory effects. The environmental concentrations of the primary pollutants were 2.0 mg/m^3 respirable dust (<10 μm MMAD), 0.2 ppm NO_2, 12 ppm CO, and 0.3 ppm formaldehyde. The investigators reported a statistically significant decline in FVC and FEV_1 over the workshift in both the diesel-exposed and comparisons group. Current smokers had greater decrements in FVC, FEV_1, and FEF_{50} than exsmokers and nonsmokers. There was a marked disparity between the ages and the time spent underground for the two study groups. Diesel-exposed miners were about 15 years younger and had worked underground for 15 fewer years (4.8 versus 20.7 years) than miners not exposed to diesel exhaust. The significance of these differences between the populations studied on the results is difficult to ascertain.

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

Gamble et al. (1978) measured preshift FEV_1 and FVC in 187 salt miners and obtained peak flow forced expiratory flow rates at 25, 50, and 75% of FVC (FEF_{25}, FEF_{50}, or FEF_{75}). Postshift pulmonary function values were determined from total lung capacity and flows at preshift percentages of FVC. The miners were exposed to mean NO_2 levels of 1.5 ppm and mean respirable particulate levels of 0.7 mg/m^3. No statistically significant changes were found between changes in pulmonary function and in NO_2 and respirable particles combined. Slopes of the regression of NO_2 and changes in FEV_1, FEF_{25}, FEF_{50}, and FEF_{75} were significantly different from zero. The authors concluded that these small reductions in pulmonary function were attributable to variations in NO_2 within each of the five salt mines that contributed to the cohort.

Gamble et al. (1987a) investigated the acute effects of diesel exhaust in 232 workers in four diesel bus garages using an acute respiratory questionnaire and before and after workshift

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

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

5.1.1.1.3. ***Immunological effects.*** The likelihood that diesel exhaust can induce reactive airway disease in humans was first reported by Wade and Newman (1993). They identified three

railroad workers who developed asthma following either a single exposure or a series of short-term exposures to high concentrations of DE. Asthma diagnosis was based on symptoms, pulmonary function tests, and measurement of airway hyperreactivity to methacholine or exercise. Exposure occurred as a result of train crews riding in locomotive units trailing immediately behind the lead engine. Although the individuals had worked for the railroad for many years and presumably had been chronically exposed to lower levels of exhaust, the symptoms developed following these subacute incidents. Unfortunately, exposure levels were not measured.

In an attempt to evaluate the potential allergenic effects of DE in humans Diaz-Sanchez and associates carried out a series of clinical investigations. In the first of these (Diaz-Sanchez et al., 1994), humans were challenged by spraying either saline or 0.30 mg DPM into their nostrils. This dose was considered equivalent to total exposure on 1-3 average days in Los Angeles, but could occur acutely in certain nonoccupational settings such as sitting at a busy bus stop or in an express tunnel. Enhanced IgE levels were noted in nasal lung lavage cells in as little as 24 h, with peak production observed 4 days after DPM challenge. The effects semed to be somewhat isotype-specific, because in contrast to IgE results, DPM challenge had no effect on the levels of IgG, IgA, IgM, or albumin. The selective enhancement of local IgE production was demonstrated by a dramatic increase in IgE-secreting cells. Takenaka et al. (1995) reported that DPM extracts enhanced IgE production from purified human B cells. Interleukin-4 plus monoclonal antibody-stimulated IgE production was enhanced 20% to 360% by the addition of DPM extracts over a period of 10-14 days. DPM extracts themselves did not induce IgE production or synergize with interleukin-4 alone to induce IgE from purified B cells, suggesting that the extracts were enhancing ongoing IgE production rather than inducing germline transcription or isotype switching. The authors concluded that enhancement of IgE production in the human airway resulting from the organic fraction of DPM may be an important factor in the increase of allergic airway disease occurring throughout this century.

Although direct effects of DPM on B-cells have been demonstrated by in vitro studies, it was considered likely that other cells regulating the IgE response may also be affected. Cytokine production was therefore measured in nasal lung lavage cells challenged with DPM (Diaz-Sanchez et al., 1996). Before challenge, most subjects' nasal lavage cells had detectable levels of only interferon-*gamma*, IL-2, and IL-13 *m*RNA. After challenge, the cells produced readily detectable levels of *m*RNA for IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and interferon *gamma.* In addition, all levels of cytokine *m*RNA were increased. Although the cells in the nasal lavage before and after challenge do not necessarily represent the same ones either in number or type, the broad increase in cytokine production was not simply the result of an increase in T cells

recovered in the lavage fluid. On the basis of these findings it was concluded that the increase in cytokine expression after exposure to DPM can be predicted to contribute to enhanced local IgE production and thus play a role in pollutant-induced airway diaease.

In a continuation of the previous studies, the combined effects of intranasal challenge with DPM plus ragweed allergen were investigated. Subjects were selected on the basis of being allergic to ragweed. They were tested with doses of ragweed antigen sufficient to induce a detectable response, 0.3 mg DPM, or ragweed allergen plus DPM combined (Diaz-Sanchez et al., 1997). As compared with challenge by ragweed alone, challenge with both DPM and ragweed allergen induced markedly higher ragweed-specific IgE, but not total IgE levels. This synergy between DPM and natural allergen exposure may be a key feature in increasing allergen-induced respiratory allergic disease.

In the available exposure studies, considerable variability is reported in diesel exhaust detection threshold. The odor scales described in some of these studies have no general use at present because they are not objectively defined; however, the studies do clearly indicate substantial interindividual variability in the ability to detect odor and the level at which it becomes objectionable. Much of what is known about the acute effects of diesel exhaust comes from case reports that lack clear measurements of exposure concentrations. The studies of pulmonary function changes in exposed humans have looked for changes occurring over a workshift or after a short-term exposure. The overall conclusion of these studies is that reversible changes in pulmonary function in humans can occur in relation to diesel exhaust exposure, although it is not possible to relate these changes to specific exposure levels. Based on the report by Wade and Newman (1993), reversible airflow obstruction and a syndrome consistent with asthma are possible following acute, high-level exposure to diesel exhaust. Recent studies by Diaz-Sanchez and co-workers have provided data indicating that DPM is a likely factor in the increasing incidence of allergic hypersensitivity. They have also shown that effects are due primarily to the organic fraction and that DPM synergizes with known allergens to increase their effectiveness.

5.1.1.2. *Long-Term Exposures*

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

Battigelli et al. (1964) measured several indices of pulmonary function, including vital capacity, FEV_1, peak flow, nitrogen washout, and diffusion capacity in 210 locomotive repairmen exposed to diesel exhaust in three engine houses. The average exposure of these locomotive repairmen to diesel exhaust was 9.6 years. When compared with a control group

matched for age, body size, "past extrapulmonary medical history" (no explanation given), and job status (154 railroad yard workers), no significant clinical differences were found in pulmonary function or in the prevalence of dyspnea, cough, or sputum between the diesel exhaust-exposed and nonexposed groups. Exposure to the diesel exhaust showed marked seasonal variations because the doors of the engine house were open in the summer and closed in the winter. For the exposed group, the maximum daily workplace concentrations of air pollutants measured were 1.8 ppm NO_2, 1.7 ppm total aldehydes, 0.15 ppm acrolein, 4.0 ppm SO_2, and 5.0 ppm total hydrocarbons. The concentration of airborne particles was not reported.

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

Purdham et al. (1987) evaluated respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations who were exposed to both diesel and gasoline exhausts and in a control group of 11 on-site office workers. Twenty-four percent of the exposed group and 36% of the controls were smokers. If a particular symptom was considered to be influenced by smoking, smoking status was used as a covariate in the logistic regression analysis; pack-years smoked was a covariate for lung function indices. The frequency of respiratory symptoms was not significantly different between the two groups; however, baseline pulmonary function measurements were significantly different. The latter comparisons were measured by multiple regression analysis using the actual (not percentage predicted) results and correcting for age, height, and pack-years smoked. The stevedores had significantly lower FEV_1, FEV_1/FVC, FEF_{50}, and FEF_{75} ($p<0.021$, $p<0.023$, $p<0.001$, and $p<0.008$, respectively) but not FVC. The results from the stevedores were also compared with those obtained from a study of the respiratory health status of Sydney, Nova Scotia, residents. These comparisons showed that the dock workers had higher FVC, similar FEV_1, but lower FEV_1/FVC and flow rates than the

residents of Sydney. Based on these consistent findings, the authors concluded that the lower baseline function measurements in the stevedores provided evidence of an obstructive ventilatory defect but caution in interpretation was warranted because of the small sample size. There were no significant changes in lung function over the workshift, nor was there a difference between the two groups. The stevedores were exposed to significantly ($p<0.04$) higher concentrations of particulate matter (0.06 to 1.72 mg/m^3, mean 0.50 mg/m^3) than the controls (0.13 to 0.58 mg/m^3, mean not reported). Exposures of stevedores to SO_2, NO_2, aldehydes, and PAHs were very low; occasional CO concentrations in the 20 to 100 ppm range could be detected for periods up to 1 h in areas where blockers were chaining gasoline-powered vehicles.

Additional epidemiological studies on the health hazards posed by exposure to diesel exhaust have been conducted for mining operations. Reger et al. (1982) evaluated the respiratory health status of 823 male coal miners from six diesel-equipped mines compared with 823 matched coal miners not exposed to diesel exhaust. The average tenure of underground work for the underground miners and their controls was only about 5 years; on average, the underground workers in diesel mines spent only 3 of those 5 years underground in diesel-use mines. Underground miners exposed to diesel exhaust reported a higher incidence of symptoms of cough and phlegm but proportionally fewer symptoms of moderate to severe dyspnea than their matched counterparts. These differences in prevalence of symptoms were not statistically significant. The diesel-exposed underground miners, on the average, had lower FVC, FEV_1, FEF_{50}, FEF_{75}, and FEF_{90} but higher peak flow and FEF_{25} than their matched controls. These differences, however, were not statistically significant. Health indicators for surface workers and their matched controls were directionally the same as for matched underground workers. There were no consistent relationships between the findings of increased respiratory symptoms, decreased pulmonary function, smoking history, years of exposure, or monitored atmosphere pollutants (NO_x, CO, particles, and aldehydes). Mean concentrations of NO_x at the six mines ranged from 0 to 0.6 ppm for short-term area samples, 0.13 to 0.28 ppm for full-shift personal samples, and 0.03 to 0.80 for full-shift area samples. Inhalable particles (less than 10 μm MMAD) averaged 0.93 to 2.73 mg/m^3 for personal samples and 0 to 16.1 mg/m^3 for full shift area samples. Ames et al. (1984), using a portion of the miners studied by Reger, examined 280 diesel-exposed underground miners initially in 1977 and again in 1982. Each miner in this group had at least 1 year of underground mining work history in 1977. The control group was 838 miners with no exposure to diesel exhaust. The miners were evaluated for the prevalence of respiratory symptoms, chronic cough, phlegm, dyspnea, and changes in FVC, FEV_1, and FEF_{50}. No air monitoring data were reported; exposure to diesel exhaust gases and mine dust particles were described as very low. These authors found no decrements in pulmonary function or

increased prevalence of respiratory symptoms attributable to exposure to diesel exhaust. In fact, the 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in those exposed to diesel exhaust.

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

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

Gamble et al. (1983) investigated respiratory morbidity in 259 miners from five salt mines in terms of increased respiratory symptoms, radiographic findings, and reduced pulmonary function associated with exposure to NO_2, inhalable particles (<10 μm MMAD), or years worked underground. Two of the mines used diesel extensively; no diesels were used in one salt mine. Diesels were introduced into each mine in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt miner cohort. After adjustment for age and smoking, the salt miners showed no increased prevalence of cough, phlegm, dyspnea, or airway obstruction (FEV_1/FVC) compared with aboveground coal miners, potash miners, or blue-collar workers. The underground coal miners consistently had an elevated level of symptoms. Forced expiratory volume at 1 s, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in relation to all the comparison populations. There was, however, no association between changes in pulmonary function and years worked, estimated cumulative inhalable particles, or estimated

NO_2 exposure. The highest average exposure to particulate matter was 1.4 mg/m^3 (particle size not reported, measurement includes NaCl). Mean NO_2 exposure was 1.3 ppm, with a range of 0.17 ppm to 2.5 ppm. In a continuation of these studies, Gamble and Jones (1983) grouped the salt miners into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exhaust exposure. Average concentrations of inhalable particles and NO_2 were 0.40, 0.60, and 0.82 mg/m^3 and 0.64, 1.77, and 2.21 ppm for the three diesel exposure categories, respectively. A statistically significant concentration-response association was found between the prevalence of phlegm in the salt miners and exposure to diesel exhaust ($p<0.0001$) and a similar, but nonsignificant, trend for cough and dyspnea. Changes in pulmonary function showed no association with diesel tenure. In a comparison with the control group of nonexposed, blue-collar workers, adjusted for age and smoking, the overall prevalence of cough and phlegm (but not dyspnea) was elevated in the diesel-exposed workers. Forced expiratory volumes at 1 s and FVC were within 4% of expected, which was considered to be within the normal range of variation for a nonexposed population.

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

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

To date, no large-scale epidemiological study has looked for effects of chronic exposure to diesel exhaust on pulmonary function. In the long-term longitudinal and cross-sectional

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

Study	Description	Findings
	Acute exposures	
Kahn et al. (1988)	13 cases of acute exposure, Utah and Colorado coal miners.	Acute reversible sensory irritation, headache, nervous system effects, bronchoconstriction were reported at unknown exposures.
El Batawi and Noweir (1966)	161 workers, two diesel bus garages.	Eye irritation (42%), headache (37%), dizziness (30%), throat irritation (19%), and cough and phlegm (11%) were reported in this order of incidence by workers exposed in the service and repair of diesel-powered buses.
Battigelli (1965)	Six subjects, eye exposure chamber, three dilutions.	Time to onset was inversely related and severity of eye irritation was associated with the level of exposure to diesel exhaust.
Katz et al. (1960)	14 persons monitoring diesel exhaust in a train tunnel.	Three occasions of minor eye and throat irritation; no correlation established with concentrations of diesel exhaust components.
Hare and Springer (1971) Hare et al. (1974)	Volunteer panelists who evaluated general public's response to odor of diesel exhaust.	Slight odor intensity, 90% perceived, 60% objected; slight to moderate odor intensity, 95% perceived, 75% objected; moderate odor intensity, 100% perceived, almost 95% objected.
Linnell and Scott (1962)	Odor panel under highly controlled conditions determined odor threshold for diesel exhaust.	In six panelists, the volume of air required to dilute raw diesel exhaust to an odor threshold ranged from a factor of 140 to 475.
Ruddell et al. (1988)	Volunteers exposed to about 100 $\mu g/m^3$ DPM.	Neutrophils were increased and phagocytosis of opsonized yeast cells was reduced after exposure.
Rudell et al. (1996)	Volunteeers exposed to diesel exhaust for one hour while doing light work. Exposure concentrations uncertain.	Unpleasant smell along with irritation of eyes and nose reported. Airway resistance increased. Reduction of particle concentrtion by trapping did not affect results.
Battigelli (1965)	13 volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 h.	No significant effects on pulmonary resistance were observed as measured by plethysmography.
Wade and Newman (1993)	Three railroad workers acutely exposed to diesel exhaust.	The workers developed symptoms of asthma.
Diaz-Sanchez et al. (1994)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	Enhancement of IgE production reported due to a dramatic increase in IgE-secreting cells.
Takenaka et al. (1995)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	DPM extracts enhanced interleukin-4 plus monoclonal antibody-stimulated IgE production as much as 360%, suggesting an enhancement of ongoing IgE production rather than inducing germline transcription or isotype switching.

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

Study	Description	Findings
Diaz-Sanchez et al. (1996)	Volunteeers challenged by a nasal spray of 0.30 mg DPM.	A broad increase in cytokine expression predicted to contribute to enhanced local IgE production.
Diaz-Sanchez et al. (1997)	Ragweed-sensitive volunteers challenged by a nasal spray of 0.30 mg DPM alone or in combination with ragweed allergen.	Ragweed allergen plus DPM-stimulated ragweed-specific IgE to a much greater degree than ragweed alone, suggesting DPM may be a key feature in stimulating allergen-induced respiratory allergic disease.
	Studies of cross-shift changes	
Reger et al. (1978)	Five or more VC maneuvers by each of 60 coal miners exposed to diesel exhaust at the beginning and end of a workshift.	FEV_1, FVC, and PEFR were similar between diesel and non-diesel-exposed miners. Smokers had an increased number of decrements over shift than nonsmokers.
Ames et al. (1982)	Pulmonary function of 60 diesel-exposed compared with 90 non-diesel-exposed coal miners over workshift.	Significant workshift decrements occurred in miners in both groups who smoked; no significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed.
Jörgensen and Svensson (1970)	240 iron ore miners matched for diesel exposure, smoking, and age were given bronchitis questionnaires and spirometry pre- and postworkshift.	Among underground (surrogate for diesel exposure) miners, smokers, and older age groups, frequency of bronchitis was higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age.
Gamble et al. (1978)	200 salt miners performed before and after workshift spirometry. Personal environmental NO_2 and inhalable particle samples were collected.	Smokers had greater but not significant reductions in spirometry than ex- or nonsmokers. NO_2 but not particulate levels significantly decreased FEV1, FEF_{25}, FEF_{50}, and FEF_{75} over the workshift.
Gamble et al. (1987a)	232 workers in four diesel bus garages administered acute respiratory questionnaire and before and after workshift spirometry. Compared to lead/acid battery workers previously found to be unaffected by their exposures.	Prevalence of burning eyes, headache, difficult or labored breathing, nausea, and wheeze were higher in diesel bus workers than in comparison population.

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

Study	Description	Findings
Ulfvarson et al. (1987)	Workshift changes in pulmonary function were evaluated in crews of roll-on/ roll-off ships and car ferries and bus garage staff. Pulmonary function was evaluated in six volunteers exposed to diluted diesel exhaust, 2.1 ppm NO_2, and 0.6 mg/m^3 particulate matter.	Pulmonary function was affected during a workshift exposure to diesel exhaust, but it normalized after a few days with no exposure. Decrements were greater with increasing intervals between exposures. No effect on pulmonary function was observed in the experimental exposure study.
	Cross-sectional and longitudinal studies	
Battigelli et al. (1964)	210 locomotive repairmen exposed to diesel exhaust for an average of 9.6 years in railroad engine houses were compared with 154 railroad yard workers of comparable job status but no exposure to diesel exhaust.	No significant differences in VC, FEV_1, peak flow, nitrogen washout, or diffusion capacity or in the prevalence of dyspnea, cough, or sputum were found between the diesel exhaust-exposed and nonexposed groups.
Gamble et al. (1987b)	283 male diesel bus garage workers from four garages in two cities were examined for impaired pulmonary function (FVC, FEV_1, and flow rates). Study population with a mean tenure of 9 ± 10 years S.D. was compared to a nonexposed blue-collar population.	Analyses within the study population showed no association of respiratory symptoms with tenure. Reduced FEV_1 and FEF_{50} (but not FEF_{75}) were associated with increasing tenure. The study population had a higher incidence of cough, phlegm, and wheezing unrelated to tenure. Pulmonary function was not affected in the total cohort of diesel-exposed but was reduced with 10 or more years of tenure.
Purdham et al. (1987)	Respiratory symptoms and pulmonary function were evaluated in 17 stevedores exposed to both diesel and gasoline exhausts in car ferry operations; control group was 11 on-site office workers.	No differences between the two groups for respiratory symptoms. Stevedores had lower baseline lung function consistent with an obstructive ventilatory defect compared with controls and those of Sydney, Nova Scotia, residents. Caution in interpretation is warranted due to small sample size. No significant changes in lung function over workshift or difference between two groups.
Reger et al. (1982)	Differences in respiratory symptoms and pulmonary function were assessed in 823 coal miners from six diesel-equipped mines compared to 823 matched coal miners not exposed to diesel exhaust.	Underground miners in diesel-use mines reported more symptoms of cough and phlegm and had lower pulmonary function. Similar trends were noted for surface workers at diesel-use mines. Pattern was consistent with small airway disease but factors other than exposure to diesel exhaust thought to be responsible.

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

Study	Description	Findings
Ames et al. (1984)	Changes in respiratory symptoms and function were measured during a 5-year period in 280 diesel-exposed and 838 nonexposed U.S. underground coal miners.	No decrements in pulmonary function or increased prevalence of respiratory symptoms were found attributable to diesel exhaust. In fact, 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in miners exposed to diesel exhaust.
Attfield (1978)	Respiratory symptoms and function were assessed in 2,659 miners from 21 underground metal mines (1,709 miners) and nonmetal mines (950 miners). Years of diesel usage in the mines were surrogate for exposure to diesel exhaust.	Questionnaire found an association between an increased prevalence of cough and aldehyde exposure; this finding was not substantiated by spirometry data. No adverse symptoms or pulmonary function decrements were related to exposure to NO_2, CO, CO_2, dust, or quartz.
Attfield et al. (1982)	Respiratory symptoms and function were assessed in 630 potash miners from six potash mines using a questionnaire, chest radiographs, and spirometry. A thorough assessment of the environment of each mine was made concurrently.	No obvious association indicative of diesel exposure was found between health indices, dust exposure, and pollutants. Higher prevalences of cough and phlegm but no differences in FVC and FEV_1 were found in these diesel-exposed potash workers when compared with predicted values from a logistic model based on blue-collar staff working in nondusty jobs.
Gamble et al. (1983)	Respiratory morbidity was assessed in 259 miners in five salt mines by respiratory symptoms, radiographic findings, and spirometry. Two mines used diesels extensively, two had limited use, one used no diesels in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt mine cohort.	After adjustment for age and smoking, salt miners showed no symptoms, increased prevalence of cough, phlegm, dyspnea, or air obstruction (FEV_1/FVC) compared with aboveground coal miners, potash workers, or blue-collar workers. FEV_1, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in comparison with all the comparison populations. No changes in pulmonary function were associated with years of exposure or cumulative exposure to inhalable particles or NO_2.
Gamble and Jones (1983)	Same as above. Salt miners were grouped into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exposure.	A statistically significant dose-related association of phlegm and diesel exposure was noted. Changes in pulmonary function showed no association with diesel tenure. Age- and smoking-adjusted rates of cough, phlegm, and dyspnea were 145, 169, and 93% of an external comparison population. Predicted pulmonary function indices showed small but significant reductions; there was no dose-response relationship.

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

Study	Description	Findings
Edling and Axelson (1984)	Pilot study of 129 bus company employees classified into three diesel-exhaust exposure categories: clerks (0), bus drivers (1), and bus garage workers.	The most heavily exposed group (bus garage workers) had a fourfold increase in risk of dying from cardiovascular disease, even after correction for smoking and allowing for 10 years of exposure and 15 years or more of induction latency time.
Edling et al. (1987)	Cohort of 694 male bus garage employees followed from 1951 through 1983 was evaluated for mortality from cardiovascular disease. Subcohorts categorized by levels of exposure were clerks (0), bus drivers (1), and bus garage employees (2).	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as subcohorts with different levels of exposure.

studies, a relationship was generally observed between work in a job with diesel exposure and respiratory symptoms (such as cough and phlegm), but there was no consistent effect on pulmonary function. The interpretation of these results is hampered by lack of measured diesel exhaust exposure levels and the short duration of exposure in these cohorts. Only active workers were included in these studies. It is possible that the relationship between work in a job with diesel exposure and respiratory symptoms was due to short-term exposure.

5.1.2. Laboratory Animal Studies

Because of the large number of statistical comparisons made in the laboratory animal studies and to permit uniform, objective evaluations within and among studies, data will be reported as significantly different (i.e., $p<0.05$) unless otherwise specified. The exposure regimens used and the resultant exposure conditions employed in the laboratory animal inhalation studies are summarized in Appendix A. Other than the pulmonary function studies performed by Wiester et al. (1980) on guinea pigs during their exposure in inhalation chambers, the pulmonary function studies performed by other investigators, although sometimes unreported, were interpreted as being conducted on the following day or thereafter and not immediately following exposure.

5.1.2.1. *Acute Exposures*

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

and mortality seen in the other three tests. Aldehydes and NO_2 were considered to be the main irritants in the light load test.

Kobayashi et al. (1995) administered 1, 10, or 20 mg/kg DPM for 30 min to the nasal mucosa of guinea pigs. The administration increased nasal airway resistance, augmented increased airway resistance and nasal secretion induced by a histamine aerosol, increased vascular permeability in dorsal skin, and augmented vacular permeability induced by histamine. The increases in nasal airway resistance and secretion are considered typical responses of nasal mucosa against allergic stimulation. Similar results were reported for guinea pigs exposed via inhalation for 3 h to diesel exhaust diluted to particle concentrations of either 1 or 3.2 mg/m^3 (Kobayashi et al., 1997). These studies show that short-term exposure to diesel exhaust potently induces nasal mucosal hyperrersponsiveness in guinea pigs.

The effects of diesel exhaust particles (DPM) and their components (extracted particles and particle extracts) on the release of proinflammatory cytokines, interleukin-1, and tumor necrosis factor-alpha (TNF-*a*) by alveolar macrophages (AM) were investigated by Yang et al. (1997). Rat AM were incubated with 0, 5, 10, 20, 50, or 100 $\mu g/10^6$ AM/mL of DPM, methanol-extracted DPM, or equivalent concentrations of DPM at 37 °C for 24 h. At high concentratrions, both DPM and DPM extracts were shown to increase IL-1-like activity secreted by AM, whereas extracted particles had no effect. Neither particles, particle extracts, or extracted particles stimulated secretion of TNF*a*. DPM inhibited lipid polysaccharide (LPS)-stimulated production of IL-1 and TNF-*a*. In contrast, interferon-gamma stimulated production of IL-1 and TNF-*a*.

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

Takano et al. (1997) conducted a study designed to evaluate the effects of DPM on the manifestations of allergic asthma in mice, with emphasis on antigen-induced airway inflammation, the local expression of interleukin-5 (IL-5), macrophage colony stimulating factor (GM-CSF), IL-2 and interferon (IFN)-gamma, and the production of antigen-specific IgE and IgG. Male ICR mice were intratracheally instilled with ovalbumin (OVA), DPM, and DPM+OVA. DPM was obtained from a 4JB1-type, light-duty 2.74 L, four-cylinder Izuzu diesel engine operated at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). The OVA-group mice were instilled with 1 ug OVA at 3 and 6 weeks. The mice receiving DPM alone were instilled with 100 µg DPM weekly for 6 weeks. The OVA + DPM group received the combined

treatment in the same protocol as the OVA and the DPM groups, respectively. Additional groups were exposed for 9 weeks. DPM aggravated ovalbumin-induced airway inflammation, characterized by infiltration of eosinophils and lymphocytes and an increase in goblet cells in the bronchial epithelium. DPM in combination with antigen markedly increased IL-5 protein levels in lung tissue and bronchoalveolar lavage supernatants compared with either antigen or DPM alone. The combination of DPM and antigen induced significant increases in local expression of of IL-4, GM-CSF, and IL-2, whereas expression of of IFN-gamma was not affected. In addition, DPM exhibited adjuvant activity for the antigen-specific production of IgG and IgE. These results provide experimental evidence that DPM can enhance the manifestations of allergic asthma and suggest that DPM is implicated in the increasing prevalence of allergic asthma in recent years.

5.1.2.2. *Short-Term and Subchronic Exposures*

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

The effects of a short-term diesel exhaust exposure on arterial blood gases, pH, blood buffering, body weight changes, lung volumes, and deflation pressure-volume (PV) curves of young adult rats were evaluated by Pepelko (1982a). Exposures were 20 h/day, 7 days/week for 8 days to a concentration of 6.4 mg/m^3 particulate matter in the nonirradiated exhaust (RE) and 6.75 mg/m^3 in the irradiated exhaust (IE). In spite of the irradiation, levels of gaseous compounds were not substantially different between the two groups (Table 5-2). Body weight gains were significantly reduced in the reexposed rats and to an even greater degree in rats exposed to IE. Arterial blood gases and standard bicarbonate were unaffected, but arterial blood pH was significantly reduced in rats exposed to IE. Residual volume and wet lung weight were not affected by either exposure, but vital capacity and total lung capacity were increased significantly following exposure to RE. The shape of the deflation PV curves were nearly identical for the control, RE and IE groups.

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 10-13 weeks	1.5 0.19 µm MMD	2,100 to 2,730	6.9	0.49	—	Increase in lung wt; increase in thickness of alveolar walls; minimal species difference	Kaplan et al. (1982)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 19 weeks	0.21 1.0 4.4	140 665 2,926	— — —	— — —	— — —	No effects on lung function in rats (not done in mice); increase in PMNs and proteases and AM aggregation in both species	Mauderly et al. (1981)
Cat, Inbred, M	20 h/day 7 days/week 4 weeks	6.4	3,584	14.6	2.1	2.1	Few effects on lung function; focal pneumonitis or alveolitis	Pepelko et al. (1980a)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 4 weeks	6.4 6.8[a]	3,584 3,808	16.9 16.1[a]	2.49 2.76[a] (<0.01 ppm O_3)[a]	2.10 1.86[a]	Decreased body wt; arterial blood pH reduced; vital capacity, total lung capacities increased	Pepelko (1982a)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 4 weeks	6.8[a]	3,808	16.7	2.9 (<0.01 ppm O_3)[a]	1.9	Exposure started when animals were 4 days old; increase in pulmonary flow; bradycardia	Wiester et al. (1980)
Rat, F344, M	20 h/day 5.5 days/week 4 weeks	6.0 6.8 µm MMD	2,640	—	—	—	Macrophage aggregation; increase in PMNs; Type II cell proliferation; thickened alveolar walls	White and Garg (1981)
Guinea Pig, Hartley, M	30 min	1-2 mg DPM Intranasally	—	—	—	—	Augmented increases in nasal airway resistance and vascular permeability induced by a histamine aerosol	Kobayashi et al. (1995)
Guinea Pig, Hartley, M	3 h	1 3.2	0.5 1.6	5.9 12.9	1.4 4.4	0.13 0.34	Similar results to those reported in the previous study using intranasal challange	Kobayashi et al. (1997)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3	7,056	17.4	2.3	2.1	Increase in relative lung wt. AM aggregation; hypertrophy of goblet cells; focal hyperplasia of alveolar epithelium	Wiester et al. (1980)

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse ICR, M	6 weeks	100 µg DPM intranasally	—	—	—	—	DPM aggravated ovalbumin-induced airway inflammation and provided evidence that DPM can enhance manifestations of allergic asthma	Takano et al. (1997)
Rat, Sprague-Dawley, M	24 h	5-100 $\mu g/10^6$ AM/ml of DPM	—	—	—	—	Unchanged, but not organic-free DPM enhanced production of proinflammatory cytokines	Yang et al. (1997)

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

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

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

White and Garg (1981) studied pathologic alterations in the lungs of rats (16 exposed and 8 controls) after exposure to diesel exhaust containing 6 mg/m^3 particulate matter. Two rats from the exposed group and one rat from the control group (filtered room air) were sacrificed after each exposure interval of 6 h and 1, 3, 7, 14, 28, 42, and 63 days; daily exposures were for 20 h and were 5.5 days/week. Evidence of AM recruitment and phagocytosis of diesel particles was found at the 6-h sacrifice; after 24 h of exposure there was a focal, scattered increase in the number of Type II cells. After 4 weeks of exposure, there were morphologic changes in size, content, and shape of AM, septal thickening adjacent to clusters of AMs, and an appearance of inflammatory cells, primarily within the septa. At 9 weeks of exposure, focal aggregations of particle-laden macrophages developed near the terminal bronchi, along with an influx of accumulation of black pigment-laden alveolar macrophages (AM) throughout the lung with a slight to moderate accumulation in bronchial and carinal lymph nodes. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

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

Kaplan et al. (1982) evaluated the effects of subchronic exposure to diesel exhaust on rats, hamsters, and mice. The exhaust was diluted to a concentration of 1.5 mg/m^3 particulate matter; exposures were 20 h/day, 7 days/week. Hamsters were exposed for 86 days, rats and mice for 90 days. There were no significant differences in mortality or growth rates between exposed and control animals. Lung weight relative to body weight of 15 rats exposed for 90 days was significantly higher than the mean for a control group (15 rats). Histological examination of tissues of all three species indicated particle accumulation in the lungs and mediastinal lymph nodes. Associated with the larger accumulations, there was a minimal increase in the thickness of the alveolar walls, but the vast majority of the particles elicited no response. After 6 mo of recovery, considerable clearance of the diesel particles from the lungs occurred in all three species, as evaluated by gross pathology and histopathology. However, no quantitative estimate of clearance was provided.

Toxic effects in animals from acute exposure to diesel exhaust appear to be primarily attributable to the gaseous components (i.e., mortality from CO intoxication and lung injury

caused by cellular damage resulting from NO_2 exposure). The results from short-term exposures indicate that rats experience no to minimal lung function impairment even at diesel exhaust levels sufficiently high to cause histological and cytological changes in the lung. In subchronic studies of durations of 4 weeks or more, frank adverse health effects are not readily apparent and when found are mild and result from exposure to concentrations of about 6 mg/m^3 particulate matter and durations of exposures of 20 h/day. There is ample evidence that subchronic exposure to lower levels of diesel exhaust affects the lung, as indicated by accumulation of particles, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and thickening of alveolar walls adjacent to AM aggregates. Little evidence exists, however, that subchronic exposure to diesel exhaust impairs lung function. Recent studies have implicated the organc fraction of DPM in the induction of resoiratory allergic disease.

5.1.2.3. *Chronic Exposures*

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

Increased lung weights and lung-to-body weight ratios have been reported in rats, mice, and hamsters. These data are summarized in Table 5-4. In rats exposed for up to 36 weeks to 0.25 or 1.5 mg/m^3 particulate matter, lung wet weights (normalized to body weight) were significantly higher in the 1.5 mg/m^3 exposure group than control values after 12 weeks of exposure (Misiorowski et al., 1980). Rats and Syrian hamsters were exposed for 2 years (five 16-h periods per week) to diesel exhaust diluted to achieve concentrations of 0.7, 2.2, and 6.6 mg/m^3 particulate matter (Brightwell et al., 1986). At necropsy, a significant increase in lung weight was seen in both rats and hamsters exposed to diesel exhaust compared with controls. This finding was more pronounced in the rats in which the increase was progressive with both duration of exposure and particulate matter level. The increase was greatest at 30 mo (after the end of a 6-month observation period in the high-concentration male group where the lung weight was 2.7 times the control and at 24 mo in the high-concentration female group [3.9 times

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F; Monkey, cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MMD	7,280	11.5	1.5	0.8	No effects on growth or survival	Lewis et al. (1989)
Rat, F344, M; Guinea Pig, Hartley, M	20 h/day 5 days/week 106 weeks	0.25 0.75 1.5 0.19 µm MMD	2,650 7,950 15,900	2.7[a] 4.4[a] 7.1[a]	0.1[b] 0.27[b] 0.5[b]	— — —	Reduced body weight in rats at 1.5 mg/m^3	Schreck et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	No effect on growth	Vinegar et al. (1981a,b)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8.3 0.71 µm MMD	21,663	50.0	4.0–6.0	—	No effect on growth or mortality rates	Karagianes et al. (1981)
Rat, F344, M, F; Mouse,CD-1, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.0 0.25 µm MMD	1,592 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	No effect on growth or mortality rates	Mauderly et al. (1984, 1987a)
Rat, Wistar, F; Mouse, MMRI, F	19 h/day 5 days/week 104 weeks	4.24 0.35 µm MMD	41,891	12.5	1.5	1.1	Reduced body wts; increased mortality in mice	Heinrich et al. (1986a)
Rat, F344 M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Growth reduced at 2.2 and 6.6 mg/m^3	Brightwell et al. (1986)
Rat[c] F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11[d] 0.41[d] 1.08[d] 2.31[d] 3.72[e] 0.2–0.3 µm MMD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Concentration-dependent decrease in body weight; earlier deaths in females exposed to 3.72 mg/m^3, stabilized by 15 mo	Research Committee for HERP Studies (1988)
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/week 24 mo	0.84 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Reduced body weight in rats at 2.5 and 6.98 mg/m^3 and no effect in mice	Heinrich et al. (1995)

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mice, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	Reduced body weight in NMRI mice but not in C57BL/6N mice	
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Reduced survival in 6.33 mg/m^3 after 300 days. Body weight significantly lower at 6.33 mg/m^3	Nikula et al. (1995)

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

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

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

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	I4.2	2.3	2.8	Increased lung weight	
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Increase in lung weight was significant at 2 and 6 mg/m^3	Nikula et al. (1995)
Rat		0.8 2.5 6.98					Increased lung weight in rats and mice at 3.5 and 7 mg/m^3	Henderson et al. (1988)
Mouse		6.98 4.5						

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

control]). Heinrich et al. (1986a,b; see also Stöber, 1986) found a significant increase in wet and dry weights of the lungs of rats and mice exposed at 4.24 mg/m^3 particulate matter for 1 year in comparison with controls. After 2 years, the difference was a factor of 2 (mice) or 3 (rats). After the same exposure periods, the hamsters showed increases of 50 to 75%, respectively. Exposure to equivalent filtered diesel exhaust caused no significant effects in any of the species. Vinegar et al. (1980, 1981a,b) exposed hamsters to two levels of diesel exhaust with resultant concentrations of about 6 and 12 mg/m^3 particulate matter for 8 h/day, 7 days/week for 6 mo. Both exposures significantly increased lung weight and lung weight to.body weight ratios. The difference between lung weights of exposed and control hamsters exposed to 12 mg/m^3 particulate matter was approximately twice that of those exposed to 6 mg/m^3.

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

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

No evidence was found in the published literature that chronic exposure to diesel exhaust affected the weight of body organs other than the lung and heart (e.g., liver, kidney, spleen, or testes) (Table 5-4). Morphometric analysis of hearts from rats and guinea pigs exposed to 0.25,

0.75, or 1.5 mg/m^3 particulate matter 20 h/day, 5.5 days/week for 78 weeks revealed no significant alteration in mass at any exposure level or duration of exposure (Penney et al., 1981). The analysis included relative wet weights of the right ventricle, left ventricle, combined atria, and ratio of right to left ventricle. Vallyathan et al. (1986) found no significant differences in heart weights and the ratio of heart weight to body weight between rats exposed to 2 mg/m^3 particulate matter for 7 h/day, 5 days/week for 24 mo and their respective clean air chamber controls. No significant differences were found in the lungs, heart, liver, spleen, kidney, and testes of rats exposed for 52 weeks, 7 h/day, 5 days/week to diluted diesel exhaust containing 2 mg/m^3 particulate matter compared with their respective controls (Green et al., 1983).

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

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

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

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

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

monkeys there were no specific histopathological effects reported (see next section) although particle aggregates were reported in the distal airways, suggesting more small airway deposition.

Gross (1981) exposed rats for 20 h/day, 5.5 days/week for 87 weeks to diesel exhaust containing 1.5 mg/m^3 particulate matter. When the data were normalized (e.g., indices expressed in units of airflow or volume for each animal by its own forced expiratory volume), there were no apparent functionally significant changes occurring in the lungs at 38 weeks of exposure that might be attributable to the inhalation of diesel exhaust. After 87 weeks of exposure, functional residual capacity (FRC) and its component volumes (expiratory reserve [ER] and residual volume [RV]), maximum expiratory flow (MEF) at 40% FVC, MEF at 20% FVC, and $FEV_{0.1}$ were significantly greater in the diesel-exposed rats. An observed increase in airflow at the end of the forced expiratory maneuver when a decreased airflow would be expected from the increased FRC, ER, and RV data (the typical scenario of human pulmonary disease) showed these data to be inconsistent with known clinically significant health effects. Furthermore, although the lung volume changes in the diesel-exposed rats could have been indicative of emphysema or chronic obstructive lung disease, this interpretation was contradicted by the airflow data, which suggest simultaneous lowering of the resistance of the distal airways.

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

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

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

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

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

Pepelko et al. (1980b; 1981; see also Pepelko, 1982b) and Moorman et al. (1985) measured the lung function of adult cats chronically exposed to diesel exhaust. The cats were exposed for 8 h/day and 7 days/week for 124 weeks. Exposures were at 6 mg/m^3 for the first 61 weeks and 12 mg/m^3 from weeks 62 to 124. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes (TLC, FVC, FRC, and inspiratory capacity [IC]) and the significantly lower single-breath diffusing capacity,

coupled with normal values for dynamic ventilatory function (mechanics of breathing), indicate the presence of a lesion that restricts inspiration but does not cause airway obstruction or loss of elasticity. This pulmonary physiological syndrome is consistent with an interstitial fibrotic response that was later verified by histopathology (Plopper et al., 1983).

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

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

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

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

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

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/week 24 mo	0.8 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Bronchioalveolar hyperplasia, interstitial fibrosis in all groups. Severity and incidence increase with exposure concentration	Heinrich et al. (1995)
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	No increase in tumors. Noncancer effects not discussed	
Mouse		4.5					No increase in tumors Noncancer effects not discussed	
Rat, M, F, F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11[a] 0.41[a] 1.08[a] 2.31[a] 3.72[b]	1,373 5,117 13,478 28,829 46,336	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Inflammatory changes; Type II cell hyperplasia and lung tumors seen at >0.4 mg/m^3; shortening and loss of cilia in trachea and bronchi	Research Committee for HERP Studies (1988)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4.24	48,336	12.5	1.5	1.1	Inflammatory changes; thickened alveolar septa; bronchioloalveolar hyperplasia; emphysema (diagnostic methodology not described)	Heinrich et al. (1986a)
Mouse, NMRI, F	19 h/day 5 days/week 120 weeks	4.24	48,336	12.5	1.5	1.1	Inflammatory changes; bronchio-loalveolar hyperplasia; alveolar lipoproteinosis; fibrosis	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24	56,392	12.5	1.5	1.1	Thickened alveolar septa; AM aggregation; inflammatory changes; hyperplasia; lung tumors	Heinrich et al. (1986a)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 104 weeks	0.25 0.75 1.5 6.0	2,860 8,580 17,160 68,640	— — — —	— — — —	— — — —	Minimal response at 0.25 and ultrastructural changes at 0.75 mg/m^3; thickened alveolar membranes; cell proliferation; fibrosis at 6.0 mg/m^3; increase in PMN at 0.75 mg/m^3 and 1.5 mg/m^3	Barnhart et al. (1981, 1982) Vostal et al. (1981)

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[c] 12.0[d]	41,664 83,328	20.2 33.2	2.7 4.4	2.1 5.0	Inflammatory changes; AM aggregation; bronchiolar epithelial metaplasia; Type II cell hyperplasia; peribronchiolar fibrosis	Plopper et al. (1983) Hyde et al. (1985)
Rat, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	AM hyperplasia, epithelial hyperplasia, inflammation, septal fibrosis, bronchoalveolar metaplasia	Nikula et al. (1995)

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

AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

of recovery, the lungs of all three species contained considerably less pigment, as assessed by gross pathological and histopathological examinations.

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

Nikula et al. (1997) reevaluated the lung tissue from this study. They concluded that there were no significant differences in the amount of retained particulate matter between monkeys and rats exposed under the same conditions. The rats, however, retained a greater portion of the particulate matter in lumens of the alveolar ducts and alveoli than did the monkeys. Conversely, monkeys retained a greater portion of the particulate material in the interstitum than did rats. Aggregations of particle-laden macrophages in the alveoli were rare, and there were few signs of particle-associated inflammation in the monkeys. Minimal histopathologic lesions were detected in the interstitium. Although the lungs of the monkeys showed a marginal and significantly lesser inflammatory response than rats exposed to the same exposure regime, the results should be interpreted with caution because 2 years is near the normal lifetime for rats, but less than 10% of the normal lifespan of Cynomolgus monkeys.

Histopathological effects of diesel exhaust on the lungs of rats have been investigated by the Health Effects Research Program on Diesel Exhaust (HERP) in Japan. Both light-duty (LD) and heavy-duty (HD) diesel engines were used. The exhaust was diluted to achieve nominal concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD

only) mg/m^3 particulate matter. Rats were exposed for 16 h/day, 6 days/week for 30 mo. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 particulate matter or less. At concentrations above 0.4 mg/m^3 particulate matter, severe morphological changes were observed. These changes consisted of shortened and absent cilia in the tracheal and bronchial epithelium, marked hyperplasia of the bronchiolar epithelium, and swelling of the Type II cellular epithelium. These lesions appeared to increase in severity with increases in exhaust concentration and duration of exposure. There was no difference in the degree of changes in pulmonary pathology at the same level of concentrations between the LD and the HD series.

Histological examination of the respiratory tract of hamsters revealed significantly higher numbers of hamsters exhibiting definite proliferative changes in the lungs in the group exposed to diesel exhaust than were observed in the group exposed to particle-free diesel exhaust or clean air (Heinrich et al., 1982). Sixty percent of these changes were described as adenomatous proliferations. Exposures were for 7 to 8 h/day, 5 days/week for 104 weeks to diesel exhaust diluted to achieve a concentration of 3.9 mg/m^3 particulate matter.

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

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

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

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

The effects of diesel exhaust on the lungs of 18-week-old rats exposed to 8.3 ± 2.0 mg/m^3 particulate matter were investigated by Karagianes et al. (1981). Exposures were for 6 h/day, 5 days/week, for 4, 8, 16, or 20 mo. Histological examinations of lung tissue noted focal aggregation of particle-laden AMs, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema (diagnostic methodology not described). Lesion severity was related to length of exposure. No significant differences were noted in lesion severity among the diesel exhaust, the diesel exhaust plus coal dust (5.8 ± 3.5 mg/m^3), or the high-concentration (14.9 ± 6.2 mg/m^3) coal dust exposure groups following 20 mo of exposure.

Histological changes in the lungs of guinea pigs exposed to diluted diesel exhaust containing either 0.25, 0.75, 1.5, or 6.0 mg/m^3 particulate matter were reported by Barnhart et al. (1981, 1982). Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo resulted in an uptake of exhaust particles by three alveolar cell types (AMs, Type I cells, and interstitial macrophages)

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

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

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

Histological examinations were performed on the lungs of cats initially exposed to 6 mg/m^3 particulate matter for 61 weeks and subsequently increased to 12 mg/m^3 for Weeks 62 to 124 of exposure. Plopper et al. (1983; see also Hyde et al., 1985) concluded from the results of this study that exposure to diesel exhaust produced changes in both epithelial and interstitial tissue compartments and that the focus of these lesions in the peripheral lung was the centriacinar region where the alveolar ducts join the terminal conducting airways. This conclusion was based

on the following evidence. The epithelium of the terminal and respiratory bronchioles in exposed cats consisted of three cell types (ciliated, basal, and Clara cells) compared with only one type (Clara cells) in the controls. The proximal acinar region showed evidence of peribronchial fibrosis and bronchiolar epithelial metaplasia. Type II cell hyperplasia was present in the proximal interalveolar septa. The more distal alveolar ducts and the majority of the rest of the parenchyma were unchanged from controls. Peribronchial fibrosis was greater at the end of 6 mo in clean air following exposure, whereas the bronchiolar epithelial metaplasia was most severe at the end of exposure. Following an additional 6 mo in clean air, the bronchiolar epithelium more closely resembled the control epithelial cell population.

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

Additional means for assessing the adverse effects of diesel exhaust on the lung are to examine biochemical and cytological changes in bronchoalveolar lavage fluid (BALF) and in lung tissue. Fedan et al. (1985) performed studies to determine whether chronic exposure of rats affected the pharmacologic characteristics of the rat's airway smooth muscle. Concentration-response relationships for tension changes induced with acetylcholine, 5-hydroxytryptamine, potassium chloride, and isoproterenol were assessed in vitro on isolated preparations of airway smooth muscle (trachealis). Chronic exposure to diesel exhaust significantly increased the maximal contractile responses to acetylcholine compared with control values; exposure did not alter the sensitivity (EC_{50} values) of the muscles to the agonists. Exposures were to diesel exhaust containing 2 mg/m^3 particulate matter for 7 h/day, 5 days/week for 2 years.

Biochemical studies of BALF obtained from hamsters and rats revealed that exposures to diesel exhaust caused significant increases in lactic dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), total protein, collagen, and protease (pH 5.1) after approximately 1 year and 2 years of exposure (Heinrich et al., 1986a). These responses were generally much greater in rats than in hamsters. Exposures were to diesel exhaust

containing 4.24 mg/m^3 particulate matter for 19 h/day, 5 days/week for 120 (hamsters) to 140 (rats) weeks.

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

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

Bhatnagar et al. (1980; see also Pepelko, 1982a) evaluated changes in the biochemistry of lung connective tissue of diesel-exposed rats and mice. The mice were exposed for 8 h/day and 7 days/week for up to 9 mo to exhaust containing 6 mg/m^3 particulate matter. Total lung protein content was measured as was labeled proline and labeled leucine. Leucine incorporation is an index of total protein synthesis, although collagen is very low in leucine. Proline incorporation reflects collagen synthesis. Amino acid incorporation was measured in vivo in the rat and in short-term organ culture in mice. Both rats and mice showed a large increase in total protein (41 to 47% in rats), while leucine incorporation declined and proline incorporation was unchanged.

These data are consistent with an overall depression of protein synthesis in diesel-exposed animals and also with a relative increase in collagen synthesis compared to other proteins. The increase in collagen synthesis suggested proliferation of connective tissue and possible fibrosis (Pepelko, 1982a).

A number of reports (McClellan et al., 1986; Mauderly et al., 1987a, 1990a; Henderson et al., 1988) have addressed biochemical and cytological changes in lung tissue and BALF of rodents exposed for 7 h/day, 5 days/week for up to 30 mo at concentrations of 0, 0.35, 3.5, or 7.1 mg/m^3 particulate matter. At the lowest exposure level (0.35 mg/m^3), no biochemical or cytological changes occurred in the BALF or in lung tissue in either Fischer 344 rats or CD-1 mice. Henderson et al. (1988) provide considerable time-course information on inflammatory events taking place throughout a chronic exposure. A chronic inflammatory response was seen at the two higher exposure levels in both species, as evidenced by increases in inflammatory cells (macrophages and neutrophils), cytoplasmic and lysosomal enzymes (lactate dehydrogenase, glutathione reductase, and β-glucuronidase), and protein (hydroxyproline) in BALF. Analysis of lung tissue indicated similar changes in enzyme levels as well as an increase in total lung collagen content. After 18 mo of exposure, lung tissue glutathione was depleted in a concentration-dependent fashion in rats but was slightly increased in mice. Lavage fluid levels of glutathione and glutathione reductase activity increased in a concentration-dependent manner and were higher in mice than in rats. Rats exposed for 24 mo to diesel exhaust (3.5 mg/m^3 particulate matter) had a fivefold increase in the bronchoconstrictive prostaglandin PGF2α and a twofold increase in the inflammatory leukotriene LTB4. In similarly exposed mice, there was a twofold increase in both parameters. These investigators concluded that the release of larger amounts of such mediators of inflammation from the alveolar phagocytic cells of rats accounted for the greater fibrogenic response seen in that species.

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

Heinrich et al. (1995) reported on bronchoalveolar lavage in animals exposed for 24 mo and found exposure-related increases in lactate dehydrogenase, β-glucuronidase, protein, and hydroxyproline in groups exposed to 2.5 or 7 mg/m^3, although detailed data are not presented. Lavage analyses were not carried out in concurrent studies in mice.

Further effects of exposure to diesel exhaust on pulmonary cytology and lung biochemistry may be found in Section 5.1.2.3.

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

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

Several studies have been conducted investigating the effect of inhaled diesel exhaust on the deposition and fate of inert tracer particles or diesel particles themselves. Lung clearance of deposited particles occurs in two distinct phases: a rapid phase (hours to days) from the tracheobronchial region via the mucociliary escalator and a much slower phase (weeks to mo) from the nonciliated pulmonary region via primarily but not solely AMs. Battigelli et al. (1966) reported impaired tracheal mucociliary clearance in vitro in excised trachea from rats exposed for single or repeated exposures of 4 to 6 hours at two dilutions of diesel exhaust that resulted in exposures of approximately 8 and 17 mg/m^3 diesel particles. The exposure to 17 mg/m^3 resulted in decreased clearance after a single exposure as well as after a cumulative exposure of 34 or 100 hours. Clearance was reduced to a lesser extent and in fewer tracheas from animals exposed to 8 mg/m^3 for a cumulative exposure of 40 hours. Lewis et al. (1989) found no difference in the

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

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

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

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

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.25 µm MDD	1,593 15,925 31,850	2.9 16.5 29.7	0.1 0.3 0.7	— — —	No changes in tracheal mucociliary clearance after 6, 12, 18, 24, or 30 mo of exposure; increases in lung clearance half-times as early as 6 mo at 7.0 mg/m^3 level and 18 mo at 3.5 mg/m^3 level; no changes seen at 0.35 mg/m^3 level; after 24 mo of diesel exposure, long-term clearance half-times were increased in the 3.5 and 7.0 mg/m^3 groups	Wolff et al. (1987)
Rat, F344/Crl, M	7 h/day 5 days/week 24 mo	3.49	12,704	9.8	1.2	—	Doubling of long-term clearance half-time for clearance of 1.0 µm alumino-silicate particles. Less effect on clearance in animals with experimentally induced emphysema	Mauderly et al. (1990a)
MICROBIAL-INDUCED MORTALITY								
Mice, CD-1, F	—	—	—	—	—	—	No change in mortality in mice exposed intratracheally to 100 µg of DPM prior to exposure to aerosolized *Streptococcus* sp.	Hatch et al. (1985)
Mice CD-1, F	7 h/day 5 days/week 4, 12, or 26 weeks	2.0 0.23–0.36 µm MDD	280-1,820	11.5	1.5	0.8	Mortality similar at each exposure duration when challenged with Ao/PR/8/34 influenza virus; in mice exposed for 3 and 6 mo, but not 1 mo, there were increases in the percentages of mice having lung consolidation, higher virus growth, depressed interferon levels, and a fourfold reduction in hemagglutinin antibody levels	Hahon et al. (1985)
Mice, CR/CD-1, F	8 h/day 7 days/week 2 h up to 46 weeks	5.3 to 7.9	11-20,350	19 to 22	1.8 to 3.6	0.9 to 2.8	Enhanced susceptibility to lethal effects of *S. pyogenes* infections at all exposure durations (2 and 6 h; 8, 15, 16, 307, and 321 days); inconclusive results with *S. typhimurium* because of high mortality rates in controls; no enhanced mortality when challenged with A/PR8-3 influenza virus	Campbell et al. (1980, 1981)

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

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

clearance of $^{59}Fe_3O_4$ particles (1.5 μm MMAD, σg 1.8) 1 day after dosing control and diesel exhaust-exposed rats (2 mg/m^3, 7 h/day, 5 days/week for 8 weeks).

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

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

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

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

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

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

Wolff et al. (1987) investigated alterations in DPM clearance from the lungs of rats chronically exposed to diesel exhaust at 0, 0.35, 3.5, or 7.0 mg/m^3 DPM for 7 h/day, 5 days/week for up to 24 mo. Progressive increases in lung burdens were observed over time in the 3.5 and 7.0 mg/m^3 exposure groups. Levels of DPM in terms of milligrams per lung were 0.60, 11.5, and 20.5 after 24 mo of exposure at the 0.35, 3.5, or 7.0 mg/m^3 exposure levels, respectively. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of $^{67}Ga_2O_3$ (0.1 μm MMD) as early as 6 mo at the 7.0 mg/m^3 level and 18 mo at the 3.5 mg/m^3 level; no significant changes were seen at the 0.35 mg/m^3 level. Rats inhaled fused aluminosilicate particles (2 μm MMAD) labeled with ^{134}Cs after 24 mo of diesel exhaust exposure; long-term clearance half-times were 79, 81, 264, and 240 days for the 0, 0.35, 3.5, and 7.0 mg/m^3 groups, respectively. Differences were significant between the control and the 3.5 and 7.0 mg/m^3 groups ($p < 0.01$).

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

Mauderly et al. compared the effects of diesel exhaust in normal lungs with rats in which emphysema had been induced experimentally by instillation of elastase 6 weeks before diesel exhaust exposures. The rats were exposed to 3.5 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Measurements included histopathology, clearance, pulmonary function, lung lavage, and immune response. In the rats that were not pretreated with elastase, there was a significant reduction in the number of macrophages recovered by pulmonary lavage in contrast to the increases in macrophages reported by Strom (1984) and Henderson et al. (1988). The half-time

of the slow phase of clearance of inhaled, 1 μm, monodisperse particles was doubled in the exposure animals without elastase pretreatment. The elastase pretreatment did not affect clearance in unexposed animals but significantly reduced the effect of diesel. The clearance half-time was significantly less in elastase-pretreated, diesel-exposed animals than in diesel-exposed normal animals. Many other effects measured in this study were also less affected by diesel exposure in elastase-treated animals. Measurements of lung burden of DPM showed that elastase-pretreated animals accumulated less than half as much DPM mass as normal animals exposed at the same time, suggesting that the difference in effect could be explained by differences in dose to the lung.

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

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

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

exposure. The increase in AM counts was much larger after exposure to 1.5 mg/m^3 DPM for 6 mo than after exposure to 0.75 mg/m^3 for 1 year, although the total mass (calculated as C × T) of deposited particulate burden was the same. These data suggested to the authors that the number of lavaged AM was proportional to the mass influx of particles rather than to the actual DPM burden in the lung. These results further implied that there may be a threshold for the rate of mass influx of DPM into the lungs of rats above which there was an increased recruitment of AMs. Henderson et al. (1988) reported similar findings of significant increases of AMs in rats and mice exposed to 7.1 mg/m^3 DPM for 18 and 24 mo, respectively, for 7 h/day, 5 days/week, but not at concentrations of 3.5 or 0.35 mg/m^3 for the same exposure durations. Chen et al. (1980), using an exposure regimen of 0.25 and 1.5 mg/m^3 DPM for 2 mo and 20 h/day and 5.5 days/week, found no significant changes in absolute numbers of AMs from guinea pig BALF nor did Castranova et al. (1985) in rat BALF following exposure to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 2 years.

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

The integrity of pulmonary defense mechanisms can also be ascertained by assessing if exposure to diesel exhaust affects the colonization and clearance of pathogens and alters the challenged animals' response to respiratory tract infections. Campbell et al. (1980, 1981) exposed mice to diesel exhaust followed by infectious challenge with *Salmonella typhimurium, Streptococcus pyogenes*, or A/PR8-3 influenza virus and measured microbial-induced mortality. Exposures to the diesel exhaust were to 6 mg/m^3 DPM for 8 h/day, 7 days/week for up to 321 days. Exposure to the diesel exhaust resulted in enhanced susceptibility to the lethal effects of *S. pyogenes* infection at all exposure durations (2 h, 6 h; 8, 15, 16, 307, and 321 days). Tests with

S. typhimurium were inconclusive because of the high mortality rates in the controls. The mice exposed to diesel exhaust did not exhibit an enhanced mortality when challenged with the influenza virus. Hatch et al. (1985) found no changes in the susceptibility of mice to Group C *Streptococcus* sp. infection following intratracheal injection of 100 μg of DPM suspended in unbuffered saline.

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

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

5.1.2.3.5. ***Effects on the immune system.*** The effects of diesel exhaust on the immune system of guinea pigs were investigated by Dziedzic (1981). Exposures were to 1.5 mg/m^3 DPM for 20 h/day, 5.5 days/week for up to 8 weeks. There was no effect of diesel exposure when compared

with matched controls for the number of B and T lymphocytes and null cells isolated from the tracheobronchial lymph nodes, spleen, and blood. Cell viability as measured by trypan blue exclusion was comparable between the exposed and control groups. The results of this study and others on the effects of exposure to diesel exhaust on the immune system are summarized in Table 5-8.

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

Bice et al. (1985) evaluated whether or not exposure to diesel exhaust would alter antibody immune responses induced after lung immunization of rats and mice. Exposures were to 0.35, 3.5, or 7.1 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Chamber controls and exposed animals were immunized by intratracheal instillation of sheep red blood cells (SRBC) after 6, 12, 18, or 24 mo of exposure. No suppression in the immune response occurred in either species. After 12, 18, and 24 mo of exposure, the total number of anti-SRBC IgM antibody forming cells (AFCs) was elevated in rats, but not in mice, exposed to 3.5 or 7.1 mg/m^3 DPM; after 6 mo of exposure, only the 7.1 mg/m^3 level was found to have caused this response in rats. The number of AFC per 10^6 lymphoid cells in lung-associated lymph nodes and the levels of specific IgM, IgG, or IgA in rat sera were not significantly altered. The investigators concluded that the increased cellularity and the presence of DPM in the lung-associated lymph nodes had only a minimal effect on the immune and antigen filtration function of these tissues. Takafuji et al. (1987) evaluated the IgE antibody response of mice inoculated intranasally at intervals of 3 weeks with varying doses of a suspension of DPM in ovalbumin.

Antiovalbumin IgE antibody titers, assayed by passive cutaneous anaphylaxis, were enhanced by doses as low as 1 μg of particles compared with immunization with ovalbumin alone.

The inhalation of diesel exhaust appeared to have only minimal effects on the immune status of rats and guinea pigs. Conversely, intranasally delivered doses as low as 1 μg of DPM exerted an adjuvant activity for IgE antibody production in mice. Further studies of the effects of

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

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, BDFI, F	—	—	—	—	—	—	Intranasally delivered doses of DPM as low as 1 µg exerted an adjuvant activity for IgE antibody production	Takafuji et al. (1987)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 4 or 8 weeks	1.5 0.19 µm MDD	660 or 7,280	7.5	—	—	No alterations in numbers of B, T, and null lymphocytes or cell viability among lymphocytes isolated from tracheobronchial lymph nodes, spleen, or blood	Dziedzic (1981)
Rat, F344, M	7 h/day 5 days/week 52 or 104 weeks	2.0 0.23–0.36 µm MDD	3,640 or 7,280	11.5	1.5	0.8	Neither humoral immunity (assessed by enumerating antibody-producing cells) nor cellular immunity (assessed by the lymphocyte blast transformation assay) were markedly affected	Mentnech et al. (1984)
Rat, F344; Mouse, CD-1	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 µm MDD	1,274 12,740 25,480	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Total number of anti-sheep red blood cell IgM AFC in the lung-associated lymph nodes was elevated in rats exposed to 3.5 or 7.0 mg/m^3 DPM (no such effects in mice); total number of AFC per 10^6 lymphoid cells in lung-associated lymph nodes and level of specific IgM, IgG, or IgA in rat sera were not altered	Bice et al. (1985)

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

diesel exhaust on the immune system are needed to clarify the impact of such variables as route of exposure, species, dose, and atopy.

5.1.2.3.6. ***Effects on the liver***. Meiss et al. (1981) examined alterations in the hepatic parenchyma of hamsters by using thin-section and freeze-fracture histological techniques. Exposures to diesel exhaust were for 7 to 8 h/day, 5 days/week, for 5 mo at about 4 or 11 mg/m^3 DPM. The livers of the hamsters exposed to both concentrations of diesel exhaust exhibited moderate dilatation of the sinusoids, with activation of the Kupffer cells and slight changes in the cell nuclei. Fatty deposits were observed in the sinusoids, and small fat droplets were occasionally observed in the peripheral hepatocytes. Mitochondria often had a loss of cristae and exhibited a pleomorphic character. Giant microbodies were seen in the hepatocytes, which were moderately enlarged, and gap junctions between hepatocytes exhibited a wide range in structural diversity. The results of this study and others on the effect of exposure of diesel exhaust on the liver of laboratory animals are summarized in Table 5-9.

Green et al. (1983) and Plopper et al. (1983) reported no changes in liver weights of rats exposed to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 52 weeks or of cats exposed to 6 to 12 mg/m^3, 8 h/day, 7 days/week for 124 weeks.

The use of light and electron microscopy revealed that long-term inhalation of varying high concentrations of diesel exhaust caused numerous alterations to the hepatic parenchyma of guinea pigs. A less sensitive index of liver toxicity, increased liver weight, failed to denote an effect of diesel exhaust on the liver of the rat and cat following long-term exposure to diesel exhaust. These results are too limited to understand potential impacts on the liver.

5.1.2.3.7. ***Blood and cardiovascular systems***. Several studies have evaluated the effects of diesel exhaust exposure on hematological and cardiovascular parameters of laboratory animals. These studies are summarized in Table 5-10. Standard hematological indices of toxicological effects on red and white blood cells failed to denote dramatic and consistent responses. Erythrocyte (RBC) counts were reported as being unaffected in cats (Pepelko and Peirano, 1983), rats and monkeys (Lewis et al., 1989), guinea pigs and rats (Penney et al., 1981), and rats (Karagianes et al., 1981); lowered in rats (Heinrich et al., 1982); and elevated in rats (Research Committee for HERP Studies, 1988; Brightwell et al., 1986). Mean corpuscular volume was significantly increased in monkeys, 69 versus 64 (Lewis et al., 1989), and hamsters (Heinrich et al., 1982) and lowered in rats (Research Committee for HERP Studies, 1988). The only other parameters of erythrocyte status and related events were lowered mean corpuscular hemoglobin

Table 5-9. Effects of exposure to diesel exhaust on the liver of laboratory animals

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

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

Table 5-10. Effects of exposure to diesel exhaust on the hematological and cardiovascular systems of laboratory animals

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2 0.23–0.36 µm MDD	7,280	11.5	1.5	0.8	Increased MCV	Lewis et al. (1989)
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2 0.23–0.36 µm MDD	7,280	11.5	1.5	0.8	Increase in banded neutrophils; no effect on heart or pulmonary arteries	Lewis et al. (1989) Vallyathan et al. (1986)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3[a] 6.8[b]	7,056 7,616	17.4 16.7	2.3 2.9	2.1 1.9	No effect on heart mass or ECG; small decrease in heart rate (IE only)	Wiester et al. (1980)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 75 weeks	3.9 0.1 µm MDD	10,238-11,700	18.5	1.2	3.1	At 29 weeks, lower erythrocyte count; increased MCV; reduced leukocyte count	Heinrich et al. (1982)
Rat, F344; Guinea Pig, Hartley	20 h/day 5.5 days/week 78 weeks	0.25 0.75 1.5 0.19 µm MDD	2,145 6,435 12,870	3.0 4.8 6.9	0.11 0.27 0.49	— — —	No changes in heart mass or hematology at any exhaust level or duration of exposure in either species	Penney et al. (1981)
Rat, Wistar, M	6 h/day 5 days/week 78 weeks	8.3 0.71 µm MDD	19,422	50.0	4-6	—	3% increase in COHb	Karagianes et al. (1981)
Rat, F3444/Jcl, M, F	16 h/day 6 days/week 130 weeks	0.11[c] 0.41[c] 1.08[c] 2.31[c] 3.72[d] 0.1 µm MDD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	At higher concentrations, RBC, Hb, Hct slightly elevated; MCV and mean corpuscular hemoglobin and concentration were lowered	Research Committee for HERP Studies (1988)
Rat, F344	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Increases in RBC, Hb, Hct, and WBC, primarily banded neutrophils; suggestion of an increase in prothrombin time; increased heart/body weight and right ventricular/heart ratios and decreased left ventricular contractility in 6.6 mg/m^3 group	Brightwell et al. (1986)

Table 5-10. Effects of exposure to diesel exhaust on the hematological and cardiovascular systems of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Cat, Inbred, M	8 h/day 7 days/week 124 weeks	6.0[e] 12.0[f]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	Increases in banded neutrophils; significant at 12 mo, but not 24 mo	Pepelko and Peirano (1983)

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

Key: MCV = Mean corpuscular volume.

and mean corpuscular hemoglobin concentration in rats (Research Committee for HERP Studies, 1988), a 3 to 5% increase in carboxyhemoglobin saturation in rats (Karagianes et al., 1981), and a suggestion of an increase in prothrombin time (Brightwell et al., 1986). The biological significance of these findings regarding adverse health effects is deemed to be inconsequential.

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

An important consequence of particle retention in the lungs of exposed subjects can be the development of pulmonary hypertension and cor pulmonale. Such pathology usually arises from pulmonary fibrosis or emphysema obliterating the pulmonary vascular bed or by chronic anoxia. No significant changes in heart mass were found in guinea pigs or rats exposed to diesel exhaust (Wiester et al., 1980; Penney et al., 1981; Lewis et al., 1989). Rats exposed to diesel exhaust showed a greater increase in the medial wall thickness of pulmonary arteries of differing diameters and right ventricular wall thickness; these increases, however, did not achieve statistically significant levels (Vallyathan et al., 1986). Brightwell et al. (1986) reported increased heart/body weight and right ventricular/heart weight ratios and decreased left ventricular contractility in rats exposed to 6.6 mg/m^3 DPM for 16 h/day, 5 days/week for 104 weeks.

5.1.2.3.8. ***Serum chemistry.*** A number of investigators have studied the effects of exposure to diesel exhaust on serum biochemistry and no consistent effects have been found. Such studies are summarized in Table 5-11.

The biological significance of changes in serum chemistry in female but not male rats exposed at 2 mg/m^3 DPM for 7 h/day, 5 days/week for 104 weeks (Lewis et al., 1989) is difficult to interpret. Not only were the effects noted in one sex (females) only, but the serum enzymes, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT), were elevated in the control group, a circumstance contrary to denoting organ damage in the exposed female rats. The elevations of liver-related

Table 5-11. Effects of chronic exposures to diesel exhaust on serum chemistry of laboratory animals

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

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

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

serum enzymes in the control versus the exposed female rats appear to be a random event among these aged subjects. The incidence of age-related disease, such as mononuclear cell leukemia, can markedly affect such enzyme levels, seriously compromising the usefulness of a comparison to historical controls. The serum sodium values of 144 versus 148 mmol/L in control and exposed rats, respectively, although statistically different, would have no biological import.

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

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

5.1.2.3.9. ***Effects on microsomal enzymes***. Several studies have examined the effects of diesel exhaust exposure on microsomal enzymes associated with the metabolism and possible activation of xenobiotics, especially polynuclear aromatic hydrocarbons. These studies are summarized in Table 5-12. Lee et al. (1980) measured the activities of aryl hydrocarbon hydroxylase (AHH) and epoxide hydrase (EH) in liver, lung, testis, and prostate gland of adult male rats exposed to 6.32 mg/m^3 DPM 20 h/day for 42 days. Maximal significant AHH activities (pmol/min/mg microsomal protein) occurred at different times during the exposure period, and differences between controls and exposed rats, respectively, were as follows: prostate 0.29 versus 1.31, lung 3.67 versus 5.11, and liver 113.9 versus 164.0. There was no difference in AHH activity in the testis between exposed and control rats. Epoxide hydrase activity was not significantly different from control values for any of the organs tested.

Pepelko and Peirano (1983) found no statistical differences in liver microsomal cytochrome P448-450 levels and liver microsomal AHH between control and diesel-exposed mice either at 6 and 8 mo of exposure. Small differences were noted in the lung microsomal AHH activities, but these were believed to be artifactual differences, due to increases in

Table 5-12. Effects of chronic exposures to diesel exhaust on microsomal enzymes of laboratory animals

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

Table 5-12. Effects of chronic exposures to diesel exhaust on microsomal enzymes of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m^3)	C × T ($mg \cdot h/m^3$)	CO (ppm)	NO_2 (ppm)	SO_2 (ppm)	Effects	Study
Mouse, A/J, M	8 days/week 7 days/week 26 or 35 weeks	6.0	17.4	17.4	2.3	2.1	No differences in lung and liver AHH activities and liver P-448, P-450 levels	Pepelko and Peirano (1983)

AHH = Aryl hydrocarbon hydroclase.
B[*a*]P = Benzo[*a*]pyrene.

nonmicrosomal lung protein present in the microsomal preparations. Exposures to 6 mg/m^3 DPM were for 8 h/day, 7 days/week.

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

Chen and Vostal (1981) measured the activity of AHH and the content of cytochrome P450 in the lungs and livers of rats exposed by inhalation or intraperitoneal (i.p.) injection of a dichloromethane extract of DPM. In the inhalation exposures, the exhaust was diluted to achieve concentrations of 0.75 or 1.5 mg/m^3 DPM, and the exposure regimen was 20 h/day, 5.5 days/week for up to 9 mo. The concentration of total hydrocarbons and particle-phase hydrocarbons was not reported. Parenteral administration involved repeated i.p. injections at several dose levels for 4 days. Inhalation exposure had no significant effect on liver microsomal AHH activity; however, lung AHH activity was slightly reduced after 6 mo exposure to 1.5 mg/m^3. An i.p. dose of DPM extract, estimated to be equivalent to the inhalation exposure, had no effect on AHH activity in liver or lungs. No changes were observed in cytochrome P450 contents in lungs or liver following inhalation exposure or i.p. treatment. Direct intratracheal administration of a dichloromethane DPM extract required doses greater than 6 mg/kg body weight before the activity of induced AHH in the lung was barely doubled; liver AHH activity remained unchanged (Chen, 1986).

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

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

5.1.2.3.10. ***Effects on behavior and neurophysiology.*** Studies on the effects of exposure to diesel exhaust on the behavior and neurophysiology of laboratory animals are summarized in Table 5-13. Laurie et al. (1978) and Laurie et al. (1980) examined behavioral alterations in adult and neonatal rats exposed to diesel exhaust. Exposure for 20 h/day, 7 days/week, for 6 weeks to exhaust containing 6 mg/m^3 DPM produced a significant reduction in adult spontaneous locomotor activity (SLA) and in neonatal pivoting (Laurie et al., 1978). In a follow-up study, Laurie et al. (1980) found that shorter exposure (8 h/day) to 6 mg/m^3 DPM also resulted in a reduction of SLA in adult rats. Laurie et al. (1980) conducted additional behavioral tests on adult rats exposed during their neonatal period. For two of three exposure situations (20 h/day for 17 days postparturition, or 8 h/day for the first 28 or 42 days postparturition), significantly lower SLA was observed in the majority of the tests conducted on the adults after week 5 of measurement. When compared with control rats, adult 15-month-old rats that had been exposed as neonates (20 h/day for 17 days) also exhibited a significantly slower rate of acquisition of a bar-pressing task to obtain food. The investigators noted that the evidence was insufficient to determine whether

Table 5-13. Effects of chronic exposures to diesel exhaust on behavior and neurophysiology

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

SLA = Spontaneous locomotor activity.

the differences were the result of a learning deficit or due to some other cause (e.g., motivational or arousal differences).

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

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

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

5.1.2.3.11. ***Effects on reproduction and development.*** Studies of the effects of exposure to diesel exhaust on reproduction and development are summarized in Table 5-14. Twenty rats were exposed 8 h/day on days 6 through 15 of gestation to diluted diesel exhaust containing 6 mg/m^3 DPM (Werchowski et al., 1980a,b; Pepelko and Peirano, 1983). There were no signs of maternal toxicity or decreased fertility. No skeletal or visceral teratogenic effects were observed in 20-day-old fetuses (Werchowski et al., 1980a). In a second study, 42 rabbits were exposed to 6 mg/m^3 DPM for 8 h/day, on gestation days 6 through 18. No adverse effects on body weight gain or fertility were seen in the does exposed to diesel exhaust. No visceral or skeletal developmental abnormalities were observed in the fetuses (Werchowski et al., 1980b).

Pepelko and Peirano (1983) evaluated the potential for diesel exhaust to affect reproductive performance in mice exposed from 100 days prior to exposure throughout maturity of the F_2 generation. The mice were exposed for 8 h/day, 7 days/week to 12 mg/m^3 DPM. In general, treatment-related effects were minimal. Some differences in organ and body weights

Table 5-14. Effects of chronic exposures to diesel exhaust on reproduction and development in laboratory animals

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

were noted, but overall fertility and survival rates were not altered by exposure to diesel exhaust. The only consistent change, an increase in lung weights, was accompanied by a gross pathological diagnosis of anthracosis. These data denoted that exposure to diesel exhaust at a concentration of 12 mg/m^3 did not affect reproduction. See Section 5.3, which reports a lack of effects of exposure to diesel exhaust on rat lung development (Mauderly et al., 1987b).

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

A significant decrease in sperm number was seen at the highest dose, but testicular weight was unaffected by the treatment.

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

5.2. COMPARISON OF HEALTH EFFECTS OF FILTERED AND UNFILTERED DIESEL EXHAUST

In four chronic toxicity studies of diesel exhaust, the experimental protocol included exposing test animals to exhaust containing no particles. Comparisons were then made between the effects caused by whole, unfiltered exhaust and those caused by the gaseous components of the exhaust. Concentrations of components of the exposure atmospheres in these four studies are given in Table 5-15.

Heinrich et al. (1982) compared the toxic effects of whole and filtered diesel exhaust on hamsters and rats. Exposures were for 7 to 8 h/day and 5 days/week. Rats exposed for 24 mo to either whole or filtered exhaust exhibited no significant changes in respiratory frequency,

Table 5-15. Composition of exposure atmospheres in studies comparing unfiltered and filtered diesel exhaust[a]

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

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

respiratory minute volume, compliance or resistance as measured by a whole-body plethysmography, or heart rate. In the hamsters, histological changes (adenomatous proliferations) were seen in the lungs of animals exposed to either whole or filtered exhaust; however, in all groups exposed to the whole exhaust the number of hamsters exhibiting such lesions was significantly higher than for the corresponding groups exposed to filtered exhaust or clean air. Severity of the lesions was, however, not reported.

In a second study, Heinrich et al. (1986a, see also Stöber, 1986) compared the toxic effects of whole and filtered diesel exhaust on hamsters, rats, and mice. The test animals (96 per test group) were exposed for 19 h/day, 5 days/week for 120 (hamsters and mice) or 140 (rats) weeks. Body weights of hamsters were unaffected by either exposure. Body weights of rats and mice were reduced by the whole exhaust but not by the filtered exhaust. Exposure-related higher mortality rates occurred in mice after 2 years of exposure to whole exhaust. After 1 year of exposure to the whole exhaust, hamsters exhibited increased lung weights, a significant increase in airway resistance, and a nonsignificant reduction in lung compliance. For the same time period, rats exhibited increased lung weights, a significant decrease in dynamic lung compliance, and a significant increase in airway resistance. Test animals exposed to filtered exhaust did not exhibit such effects. Histopathological examination indicated that different levels of response occurred in the three species. In hamsters, filtered exhaust caused no significant histopathological effects in the lung; whole exhaust caused thickened alveolar septa, bronchioloalveolar hyperplasia, and emphysematous lesions. In mice, whole exhaust, but not filtered exhaust, caused multifocal bronchioloalveolar hyperplasia, multifocal alveolar lipoproteinosis, and multifocal interstitial fibrosis. In rats, there were no significant morphological changes in the lungs following exposure to filtered exhaust. In rats exposed to whole exhaust, there were severe inflammatory changes in the lungs, thickened alveolar septa, foci of macrophages, crystals of cholesterol, and hyperplastic and metaplastic lesions. Biochemical studies of lung lavage fluids of hamsters and mice indicated that exposure to filtered exhaust caused fewer changes than did exposure to whole exhaust. The latter produced significant increases in lactate dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, total protein, protease (pH 5.1), and collagen. The filtered exhaust had a slight but nonsignificant effect on G6P-DH, total protein, and collagen. Similarly, cytological studies showed that while the filtered exhaust had no effect on differential cell counts, the whole exhaust resulted in an increase in leukocytes (161 ± 43.3/μL versus 55.7 ± 12.8/μL in the controls), a decrease in AM (30.0 ± 12.5 versus 51.3 ± 12.5/μL in the controls), and an increase in granulocytes (125 ± 39.7 versus 1.23 ± 1.14/μL in the controls). All values presented for this study are the mean with its standard deviation. The differences were significant for each cell

type. There was also a small increase in lymphocytes (5.81 ± 4.72 versus 3.01 ± 1.23/μL in the controls).

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

Brightwell et al. (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Three exhaust dilutions were tested, producing concentrations of 0.7, 2.2, and 6.6 mg/m^3 DPM. The test animals (144 rats and 312 hamsters per exposure group) were exposed for five 16-h periods per week for 2 years. The four exposure types were gasoline, gasoline catalyst, diesel, and filtered diesel. The results presented were limited to statistically significant differences between exhaust-exposed and control animals. The inference from the discussion section of the paper was that there was a minimum of toxicity in the animals exposed to filtered diesel exhaust: "It is clear from the results presented that statistically significant differences between exhaust-exposed and control animals are almost exclusively limited to animals exposed to either gasoline or unfiltered diesel exhaust." Additional results are described in Section 5.4.

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

A comparison of the toxic responses in laboratory animals exposed to whole exhaust or filtered exhaust containing no particles demonstrates across studies that when the exhaust is sufficiently diluted to limit the concentrations of gaseous irritants (NO_2 and SO_2), irritant vapors (aldehydes), CO, or other systemic toxicants, the diesel particles are the prime etiologic agents of noncancer health effects, although additivity or synergism with the gases cannot be ruled out. These toxic responses are both functional and pathological and represent cascading sequelae of lung pathology based on concentration and species. The diesel particles plus gas exposures

produced biochemical and cytological changes in the lung that are much more prominent than those evoked by the gas phase alone. Such marked differences between whole and filtered diesel exhaust are also evident from general toxicological indices, such as decreases in body weight and increases in lung weights, pulmonary function measurements, and pulmonary histopathology (e.g., proliferative changes in Type II cells and respiratory bronchiolar epithelium, fibrosis). Hamsters, under equivalent exposure regimens, have lower levels of retained DPM in their lungs than rats and mice do and, consequently, less pulmonary function impairment and pulmonary pathology. These differences may result from lower DPM inspiration and deposition during exposure, greater DPM clearance, or lung tissue less susceptible to the cytotoxicity of deposited DPM.

5.3. INTERACTIVE EFFECTS OF DIESEL EXHAUST

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

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

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

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

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

The health effects of airborne contaminants from sources other than diesel engines may be altered in the presence of DPM by their adsorption onto the diesel particles. When adsorbed

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

There is no direct evidence that diesel exhaust interacts with other substances in an exposure environment or the physiological status of the exposed subject other than impaired resistance to respiratory tract infections. Although there is experimental evidence that gases and vapors can be adsorbed onto carbonaceous particles, enhancing the toxicity of these particles when deposited in the lung, there is no evidence for an increased health risk from such interactions with DPM under ambient urban atmospheric conditions. Likewise, there is no experimental evidence in laboratory animals that the youth or preexisting emphysema of an exposed individual enhances the risk of exposure to diesel exhaust.

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

There is some evidence indicating that species may differ in pulmonary responses to DE. Mauderly et al. (1993) compared the pulmonary histopathology of rats and mice after 18 mo of exposure to DE. There was less aggregation of macrophages. Diffuse septal thickening was noted in the mice, but there were few inflammatory cells, no focal fibrosis, little epithelial hyperplasia, and no epithelial metaplasia, as was observed in rats. Heinrich et al. (1986a) reported that wet lung weight of hamsters increased only 1.8-fold following chronic exposure to DE, compared with an increase of 3.4-fold in rats. Smaller increases in neutrophils, lactic acid dehydrogenase, collagen, and protein supported the conclusion of a lesser inflammatory response in Syrian hamsters. The histopathologic changes in the lungs of Chinese hamsters after 6 mo exposure to DE, on the other hand, was similar to that of rats (Pepelko and Peirano, 1983). Guinea pigs respond to chronic DE exposure with a well-defined epithelial proliferation, but it is based on an eosinophilic response in contrast to the neutrophil-based responses in other species. Epithelial hyperplasia and metaplasia were quite striking in the terminal and respiratory bronchioles of cats exposed for 27 mo to DE (Plopper et al., 1983). This study is of particular interest because the terminal airways of cats are more similar to those of humans than rodent species are. It should be noted, however, that exposure concentrations were very high (12 mg/m^3) for most of the period. Lewis et al. (1989) exposed rats and Cynamolgous monkeys 8

hours per day, 5 days per week for 2 years to DE at a particle concentration of 2 mg/m^3. Unfortunately, this exposure rate was sufficiently low that few effects were noted in either species other than focal accumulations of particles, primarily in the alveolar macrophages, interstitium, and lymphoid tissue. To achieve chronic exposure conditions in monkeys would also require a considerably longer exposure period. It is apparent that species do vary in their pulmonary responses to DE exposure, despite the difficulty in making direct comparisons because of differences in exposure regimes, lifespans, and pulmonary anatomy. Most species do respond, however, suggesting that humans are likely to be susceptible to induction of pulmonary pathology during chronic exposure to DE.

5.5. DOSE-RATE AND PARTICULATE CAUSATIVE ISSUES

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

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

changes in the lung may play a key role in the etiology of pulmonary tumors in exposed rats (Mauderly et al., 1990b).

Adults who have a preexisting condition that may predispose their lungs to increased particle retention (e.g., smoking or high particulate burdens from nondiesel sources), inflammation (e.g., repeated respiratory infections), epithelial proliferation (e.g., chronic bronchitis), and fibrosis (e.g., silica exposure) and infants and children, due to their developing pulmonary and immunologic systems, may have a greater susceptibility to the toxic actions of diesel exhaust. It should be noted that both the developing lung and a model of a preexisting disease state have been studied with regard to their effect on the lungs' response to diesel exhaust (Mauderly et al., 1990a, 1987b). Mauderly et al. (1987b) showed that diesel did not affect the developing lung more severely than the adult rat lung, and in fact, that clearance was faster in the younger lung. Mauderly et al. (1990a) compared the pulmonary response to inhalation of diesel exhaust in rats with elastase-induced emphysema with normal rats. They found that respiratory tract effects were not more severe in emphysematous rats and that the lung burden of particles was less in the compromised rat. These studies provide limited evidence that some factors that are often considered to result in a wider distribution of sensitivity among members of the population may not have this effect with diesel exposure. However, these studies have no counterpart in human studies and extrapolation to humans remains uncertain.

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

Current understanding suggests that much of the toxicity resulting from the inhalation of diesel exhaust relates to the carbonaceous core of the particles. Several studies on inhaled aerosols demonstrate that lung reactions characterized by an appearance of particle-laden AMs and their infiltration into the alveolar ducts, adjoining alveoli and tracheobronchial lymph nodes, hyperplasia of Type II cells, and the impairment of pulmonary clearance mechanisms are not limited to exposure to diesel particles. Such responses have also been observed in rats following the inhalation of coal dust (Lewis et al., 1989; Karagianes et al., 1986), titanium dioxide (Heinrich et al., 1995; Lee et al., 1985), carbon black (Nikula et al., 1995; Heinrich et al., 1995), titanium tetrachloride hydrolysis products (Lee et al., 1986), quartz (Klosterkotter and Buneman, 1961), volcanic ash (Wehner et al., 1986), amosite (Bolton et al., 1983), and manmade mineral fibers (Lee et al., 1988) among others. In more recent studies, animals have been exposed to carbon black that is similar to the carbon core of the diesel exhaust particle. Nikula et al. (1995) exposed rats for 24 mo to carbon black or diesel exhaust at target exposure concentrations of 2.5 and 6 mg/m^3 (exposure rates of 200 or 520 mg·h·m^{-3}/week). Both concentrations induced AM

accumulation, epithelial proliferation, inflammation, and fibrosis. They observed essentially no difference in potency of nonneoplastic or in tumor responses based on a regression analysis.

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

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

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

Particle size, volume, surface area, and composition may be the critical elements in the overload phenomenon following exposure to particles, which could explain those quantitative differences. The overloaded AMs secrete a variety of cytokines, oxidants, and proteolytic enzymes that are responsible for inducing particle aggregation and damaging adjacent epithelial tissue (Oberdörster and Yu, 1991). For a more detailed discussion of mechanism, see Chapter 10.

The principal noncancerous health hazard to humans posed by exposure to diesel exhaust is a structural or functional injury to the lung based on the laboratory animal data. Such effects are demonstrable at dose rates or cumulative doses of DPM lower than those reported to be necessary to induce lung tumors. Current knowledge indicates that the carbonaceous core of diesel particles is the major causative factor in the injury to the lung and that other factors such as the cytotoxicity of adsorbed substances on the particles also may play a role. The lung injury appears to be mediated through effects on pulmonary AMs. Because noncancerous pulmonary

effects occur at lower doses than tumor induction does in the rat, and because these effects may be cofactors in the etiology of diesel exhaust-induced tumors, noncancerous pulmonary effects must be considered in the total evaluation of diesel exhaust, notably the particulate component.

5.6. SUMMARY AND DISCUSSION

5.6.1. Effects of Diesel Exhaust on Humans

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

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

Smokers appeared to demonstrate larger workshift respiratory function decrements and increased incidents of respiratory symptoms. Acute sensory and respiratory symptoms were earlier and more sensitive indicators of potential health risks from diesel exposure than were decrements in pulmonary function. Studies on the acute health effects of exposure to diesel exhaust in humans, experimental and epidemiologic, have failed to demonstrate a consistent

pattern of adverse effects on respiratory morbidity; the majority of studies offer, at best, equivocal evidence for an exposure-response relationship. The environmental contaminants have frequently been below permissible workplace exposure limits; in those few cases where health effects have been reported, the authors have failed to identify conclusively the individual or collective causative agents in the diesel exhaust.

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

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

definitive conclusions as to the cause of any noncarcinogenic diesel exhaust effect, observed or reported.

5.6.2. Effects of Diesel Exhaust on Laboratory Animals

Laboratory animal studies of the toxic effects of diesel exhaust have involved acute, sub-chronic, and chronic exposure regimens. In acute exposure studies, toxic effects appear to have been associated primarily with high concentrations of carbon monoxide, nitrogen dioxide, and aliphatic aldehydes. In short- and long-term studies, toxic effects have been associated with exposure to the complex exhaust mixture. Effects of diesel exhaust in various animal species are summarized in Tables 5-2 to 5-14. In short-term studies, health effects are not readily apparent, and when found, are mild and result from concentrations of about 6 mg/m^3 DPM and durations of exposure approximating 20 h/day. There is ample evidence, however, that short-term exposures at lower levels of diesel exhaust affect the lung, as indicated by an accumulation of DPM, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and the thickening of alveolar walls adjacent to AM aggregation. Little evidence exists, however, from short-term studies that exposure to diesel exhaust impairs lung function. Chronic exposures cause lung pathology that results in altered pulmonary function and increased DPM retention in the lung. Exposures to diesel exhaust have also been associated with increased susceptibility to respiratory tract infection, neurological or behavioral changes, an increase in banded neutrophils, and morphological alterations in the liver.

5.6.2.1. *Effects on Survival and Growth*

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

5.6.2.2. *Effects on Pulmonary Function*

Pulmonary function impairment has been reported in rats, hamsters, cats, and monkeys exposed to diesel exhaust and included lung mechanical properties (compliance and resistance), diffusing capacity, lung volumes, and ventilatory performance (Table 5-5). The effects generally appeared only after prolonged exposures. The lowest exposure levels (expressed in terms of

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

5.6.2.3. *Histopathological and Histochemical Effects*

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

5.6.2.4. *Effects on Defense Mechanisms*

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

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

Several laboratory animal studies have indicated that exposure to DPM can reduce an animal's resistance to respiratory infections. This effect, which can occur even after only 2 or 6 h of exposure to diesel exhaust containing 5 to 8 mg/m^3 DPM, does not appear to be caused by direct impairment of the lymphoid or splenic immune systems; however, in one study of influenza virus infection, interferon levels and hemagglutinin antibody levels were adversely affected in the exposed mice. Studies on the effects of exposure to diesel exhaust or DPM on the immune system of laboratory animals have produced equivocal results (Table 5-8).

5.6.2.5. *Neurological and Behavioral Effects*

Behavioral effects have been observed in rats exposed to diesel exhaust from birth to 28 days of age (Table 5-13). Exposure caused a decreased level of spontaneous locomotor activity and a detrimental effect on learning in adulthood. In agreement with the behavioral changes was physiological evidence for delayed neuronal maturation. Exposures were to 6 mg/m^3 DPM for 8 h/day, 7 days/week from birth to about 7, 14, 21, or 28 days of age.

5.6.2.6. *Other Noncancerous Effects*

Essentially no effects (based on the weight of evidence of a number of studies) were noted for reproductive and teratogenic effects in mice, rats, rabbits, and monkeys; clinical chemistry and hematology in the rat, cat, hamster, and monkeys; and enzyme induction in the rat and mouse (Tables 5-10 through 5-12 and 5-14).

5.6.3. Comparison of Filtered and Unfiltered Diesel Exhaust

The comparison of the toxic responses in laboratory animals exposed to whole diesel exhaust or filtered exhaust containing no particles demonstrates across laboratories that diesel particles are the principal etiologic agent of noncancerous health effects in laboratory animals exposed to diesel exhaust (Table 5-15). Whether the particles act additively or synergistically with the gases cannot be determined from the designs of the studies. Under equivalent exposure regimens, hamsters have lower levels of retained DPM in their lungs than rats and mice do and consequently less pulmonary function impairment and pulmonary pathology. These differences may result from a lower intake rate of DPM, lower deposition rate and/or more rapid clearance rate, or lung tissue that is less susceptible to the cytotoxicity of DPM. Observations of a decreased respiration in hamsters when exposed by inhalation favor lower intake and deposition rates.

5.6.4. Interactive Effects of Diesel Exhaust

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

5.6.5. Summary

The principal noncancerous health hazard to humans from exposure to diesel exhaust is a structural, functional, or biochemical injury to the lung. Current knowledge indicates that the carbonaceous core of the diesel particle is the prime causative agent of lung injury. The lung injury appears to be mediated by a progressive impairment of AMs. Because noncancerous pulmonary effects occur at lower doses than those inducing tumors in rats and appear to be

cofactors in the etiology of diesel exhaust-induced tumors in rats, noncancerous pulmonary effects are relevant factors in the development of risk assessments.

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6. QUALITATIVE AND QUANTITATIVE ASSESSMENT OF NONCANCER HEALTH EFFECTS—DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

6.1. INTRODUCTION

Noncancer endpoints have been studied in detail in controlled laboratory animal studies of diesel exhaust, and the progression of events from initial particle deposition through chronic structural and functional alterations has been described. Some of these effects are seen early in the course of a lifetime exposure and progress throughout the lifetime of the animal in the absence of a tumor response. These findings raise the possibility of noncancer respiratory disease as a human health hazard of long-term exposure to diesel exhaust. This chapter presents a qualitative and quantitative assessment of the toxicological data on noncancer endpoints for diesel emissions.

The quantitative assessment of noncancer health effects from exposure to diesel exhaust emissions involves the development of an inhalation reference concentration (RfC). An RfC is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risks of deleterious noncancer effects during a lifetime. The RfC approach is based on the assumption that a threshold exists for the human population below which no effect will occur. The RfC is an estimate of a likely subthreshold concentration. To derive the RfC, the database on toxicological effects is reviewed and the most relevant and sensitive endpoints for human risk assessment are identified. The lowest-observed-adverse-effect level (LOAEL, the lowest concentration producing an adverse effect) or the no-observed-adverse-effect level (NOAEL, the highest concentration that did not produce any adverse effect) is used as the basis for deriving the RfC. The NOAEL (or LOAEL) for the database is selected after the human equivalent concentration is calculated for the exposure regimens used in the experimental studies. The NOAEL is considered to be an operational estimate of a subthreshold exposure. The human equivalent concentration of the NOAEL is then divided by the uncertainty factors to account for any uncertainties or data gaps necessary to extrapolate from the experimental conditions to a no-adverse-effect level in a chronically and continuously exposed sensitive human. Once consensus on the RfD derivation has been reached following both external and Agency peer review, the RfC is said to be verified and is made public through EPA's Integrated Risk Information System (IRIS).

The benchmark dose/concentration (BMC) approach may also be used to derive the RfC, as has been done for carbon disulfide, chlorodifluoromethane, and several other chemicals (U.S.

EPA, 1995a). The BMC approach, applies a dose-response model to the data from key studies and then uses the dose-response relationship to interpolate an exposure concentration that is predicted to result in a predefined response level, which is termed the benchmark response (BMR), such as a 10% incidence of a lesion or a 10% change in a continuous response variable (e.g., lung weight). The lower confidence limit on the concentration predicted to result in the BMR is the BMC, which is used as the basis for deriving the RfC. Methods for performing the BMC approach, as well as scientific consensus and Agency policy regarding the implementation of the BMC approach in risk assessment, are under development (U.S. EPA, 1995b; Barnes et al., 1995). Benchmark analyses like the one contained in this section for diesel exhaust may serve as the basis for deriving risk assessment values, as in the cases noted above, or as a point of comparison for values derived from the NOAEL/LOAEL approach.

The study or studies identifying the LOAEL, NOAEL, and/or BMC selected as the basis for deriving the RfC are termed the principal study or studies. The principal studies are those that identify the threshold region of the concentration-response curve and are representative of the entire database in this regard. Other studies that are pertinent to identifying the threshold for the effect are termed supporting studies. Supporting studies may provide additional evidence identifying the concentration-response relationship, the relative sensitivity of various effects or species, or the occurrence of other noncancer endpoints, such as reproductive or developmental toxicity. Principal and supporting studies used in deriving the RfC for diesel engine emissions are discussed in Sections 6.4 and 6.5, respectively, and the derivation of the RfC is discussed in Section 6.6.

6.2. DETERMINATION OF CRITICAL TARGET SITE

The noncarcinogenic effects of inhalation of diesel exhaust have been studied in many chronic and subchronic experiments in several laboratory animal species (Chapter 5). The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the phagocytosis of diesel particles by alveolar macrophages (AMs). These activated AMs release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of diesel particulate matter (DPM) increases, there are aggregations of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type II cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes. The neutrophils and AMs release mediators of inflammation and oxygen radicals, and particle-laden macrophages are functionally altered, resulting in decreased viability and impaired phagocytosis and clearance of particles. The latter series of events may result in pulmonary inflammatory, fibrotic, or emphysematous lesions.

Studies showing these effects are described in Chapter 5. Epidemiologic studies of people exposed in various occupations in which diesel engines are used provide suggestive evidence for a respiratory effect. Although detailed information describing the pathogenesis of respiratory effects in humans is lacking, the effects in human studies lend qualitative support to the findings in controlled animal studies.

The weight of evidence from the available toxicological data on diesel exhaust indicates with high confidence that inhalation of diesel exhaust can be a respiratory hazard, based on findings in multiple controlled laboratory animal studies in several species with suggestive evidence from human occupational studies. The endpoints of concern include biochemical, histological, and functional changes in the pulmonary and tracheobronchial regions. There is also some evidence for effects on respiratory system-related immune function. Although there is some suggestive evidence of liver and kidney changes in animals exposed to diesel exhaust, as well as some indication of neurotoxic effects at high concentrations, these data are inadequate to indicate that a hazard exists for these endpoints. Studies of other endpoints, including reproductive and developmental toxicity, in controlled animal exposures have shown no evidence of potential hazard.

6.3. APPROACH FOR DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

Twelve long-term (>1 year) laboratory animal inhalation studies of diesel engine emissions have been conducted. The focus of these studies has been on the respiratory tract effects in the pulmonary region. Effects in the upper respiratory tract and in other organs were not found consistently in chronic animal exposures. The research programs on the toxicology of diesel emissions at the Inhalation Toxicology Research Institute (ITRI) and the Japanese Health Effects Research Program (HERP) consisted of large-scale chronic exposures, with exposed animals being designated for the study of various endpoints and at various time points (Ishinishi et al., 1986, 1988; Mauderly et al., 1987a,b, 1988; Henderson et al., 1988; Wolff et al., 1987). Each research program is represented by multiple published accounts of results. These programs were selected as the principal basis for deriving the RfC because each contains studies that identify an LOAEL and an NOAEL for respiratory effects after chronic exposure (see Section 5.2) as well as pulmonary histopathology.

Diesel particulate matter is composed of an insoluble carbon core with a surface coating of relatively soluble organic constituents. Because macrophage accumulation, epithelial histopathology, and reduced clearance have been observed in rodents exposed to high concentrations of chemically inert particles (Morrow, 1992), it appears possible that the toxicity

of DPM results from the carbon core rather than from the associated organics. However, the organic component of diesel particles, consisting of a large number of polycyclic aromatic hydrocarbons and heterocyclic compounds and their derivatives (Chapter 2), may also play a role in the pulmonary toxicity of DPM. It is not possible to separate the carbon core from the adsorbed organics to compare the toxicity. Therefore, the whole particle was used as the dosimeter. See Chapters 5 and 10 for further details.

The use of a specific retention or physiologically based pharmacokinetic model is considered the optimum method for RfC derivation, and default approaches are described for chemicals without applicable models. A model developed by Yu and Yoon (1990) that accounts for species differences in deposition efficiency, normal and particle overload lung clearance rates, respiratory exchange rates, and particle transport to lung-associated lymph nodes was selected for development of the RfC. Because the dependence of mechanical alveolar clearance on particle lung burden in humans is not known, it was assumed in development of the model for humans that the particle overload phenomenon occurs in humans and in rats at equivalent lung burdens expressed as mass per unit surface area (Yu and Yoon, 1990). This assumption allows for the development of a diesel particle-specific human retention model and therefore allows extrapolation from the rat studies to human exposures. The model has not been extended to other species at this time because data describing the dependence of the particle overload phenomenon on lung particle burden for species other than the rat are not available. See Chapter 4 for further discussion of the model.

The input data required to run the dosimetric model include the particle size characterization expressed as mass median aerodynamic diameter (MMAD) and the geometric standard deviation (σg). In the principal and supporting studies used for the RfC derivation, these parameters are measured using different methods and are reported in different levels of detail. Simulation data presented by Yu and Xu (1986) show that across a range of MMAD and σg inclusive of the values reported in these studies, the pulmonary deposition fraction differs by no more than 20%. The minimal effect of even a large distribution of particle size on deposition probably results because the particles are still mostly in the submicron range and deposition is influenced primarily by diffusion. However, it has also been shown that the particle characteristics in a diesel exhaust exposure study depend very much on the procedures used to generate the chamber atmosphere. Especially important are the volume and temperature of the dilution gas, because of the rapid coagulation of particles. The differences reported in particle sizes and distributions in various studies likely reflected real differences in the exposure chambers as well as different analytical methods. Because the particle diameter and size distribution were not reported in the two lowest exposure concentrations in the HERP studies, it

was decided to use a default particle size of MMAD = 0.2 μm and σg = 2.3 for modeling of lung burden. For consistency, the lung burdens for the other studies were also calculated using the default particle size assumption. The difference in the human equivalent concentration using the default particle size compared with the actual reported particle size is no more than 4% in the HERP study and 19% in the ITRI study.

6.4. THE PRINCIPAL STUDIES FOR INHALATION REFERENCE CONCENTRATION DERIVATION

The experimental protocol and results for the principal studies are discussed in Chapter 5 and Appendix A and are briefly reviewed here. In studies conducted at ITRI, rats and mice were exposed to target DPM concentrations of 0, 0.35, 3.5, or 7 mg/m^3 for 7 h/day, 5 days/week for up to 30 mo (rats) or 24 mo (mice) (Mauderly et al., 1988). A total of 364 to 367 rats per exposure level were exposed and used for various studies examining different endpoints such as carcinogenicity, respiratory tract histopathology and morphometric analysis, particle clearance, lung burden of DPM, pulmonary function testing, lung biochemistry, lung lavage biochemistry and cytology, immune function, and lung cell labeling index. Subsets of animals were examined at 6, 12, 18, and 24 mo of exposure and surviving rats were examined at 30 mo. Diesel emissions from a 5.7-L engine operated on a Federal Test Procedure urban driving cycle were diluted and fed into the exposure chambers. Particle concentrations were measured daily using a filter sample, and weekly grab samples were taken to measure gaseous components including carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, and hydrocarbons. The actual DPM concentrations for the low-, medium-, and high-exposure levels were 0.353, 3.47, and 7.08 mg/m^3, respectively. Mass median diameters (geometric standard deviations) determined using an impactor/parallel flow diffusion battery were 0.262 (4.2), 0.249 (4.5), and 0.234 (4.4) for the low-, medium-, and high-exposure groups, respectively.

Lung wet weight to dry weight ratio was increased significantly in the two highest exposure groups. Qualitative descriptions of the histological results in the respiratory tract are found in Mauderly et al. (1987a, 1988), Henderson et al. (1988), and McClellan et al. (1986). Aggregates of particle-laden AMs were seen after 6 mo in rats exposed to 7 mg/m^3 DPM target concentrations, and after 1 year of exposure histological changes were seen, including focal areas of epithelial metaplasia. Fibrosis and metaplasia increased with increasing duration of exposure and were observable in the 3.5 and 7 mg/m^3 groups of rats at 24 mo. Changes in the epithelium included extension of bronchiolar cell types into the alveoli. Focal thickening of the alveolar septa was also observed. Histological effects were seen in areas near aggregations of particle-laden AMs. The severity of inflammatory responses and fibrosis was directly related to the

exposure level. In the 0.35 mg/m^3 group of rats, there was no inflammation or fibrosis. Although the mouse lungs contained higher lung burdens of DPM per gram of lung weight at each equivalent exposure concentration, there was substantially less inflammatory reaction and fibrosis than was the case in rats. Fibrosis was observed only in the lungs of mice exposed at 7 mg/m^3 DPM and consisted of fine fibrillar thickening of occasional alveolar septa.

Groups of 16 rats and mice (8/sex) were subjected to bronchoalveolar lavage after 6, 12, 18, and 24 (rats only) mo of exposure (Henderson et al., 1988). Lung wet weights were increased at 7 mg/m^3 in mice and rats at all time points and in mice at 3.5 mg/m^3 at all time points after 6 mo. An increase in lavagable neutrophils, indicating an inflammatory response in the lung, was seen at 3.5 and 7 mg/m^3 in rats and mice at most time points. An increase in protein content of the bronchoalveolar lavage fluid was observed in rats exposed to 3.5 or 7 mg/m^3 at 12 and 18 mo but not at 24 mo. Increased protein content was also seen in mice at the two higher concentrations at all time points. Increases in lavage fluid content of lactate dehydrogenase, glutathione reductase, β-glucuronidase, glutathione, and hydroxyproline were observed in rats and mice exposed to 3.5 or 7 mg/m^3 at various time points. At the lowest exposure level, no biochemical or cytological changes occurred in the lavage fluid or in lung tissue in either Fischer 344 rats or CD-1 mice.

Mauderly et al. (1988; see also McClellan et al., 1986) examined the impairment of respiratory function in rats exposed according to the protocol described above. After 24 mo of exposure to 7 mg/m^3 DPM, mean TLC, C_{dyn}, quasi-static chord compliance, and CO diffusing capacity were significantly lower than control values, and nitrogen washout and percentage of forced vital capacity expired in 0.1 s were significantly greater than control values. There was no evidence of airflow obstruction. Similar functional alterations were observed in the rats exposed to 3.5 mg/m^3 DPM, but such changes usually occurred later in the exposure period and were generally less pronounced. There were no significant decrements in pulmonary function for the 0.35 mg/m^3 group at any time during the study.

Wolff et al. (1987) investigated alterations in particle clearance from the lungs of rats in the ITRI study. Progressive increases in lung burdens were observed over time in the 3.5 and 7.0 mg/m^3 exposure groups. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of gallium oxide (0.1 μm MMAD) as early as 6 mo at the 7.0 mg/m^3 level and 18 mo at the 3.5 mg/m^3 level; no significant changes were seen at the 0.35 mg/m^3 level. Rats that inhaled fused aluminosilicate particles (2 μm MMAD) radiolabeled with cesium after 24 mo of diesel exhaust exposure showed increased clearance half-times in the 3.5 and 7.0 mg/m^3 groups.

In the HERP studies, histopathological effects of diesel exhaust on the lungs of rats were investigated (Ishinishi et al., 1986, 1988). In this study, both light-duty (LD, 1.8-L) and heavy-duty (HD, 11-L) diesel engines were operated under constant velocity and load conditions. The exhaust was diluted to achieve target concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD only) mg/m^3 DPM. Particle concentrations were determined by filter samples. Actual concentrations were 0.11, 0.41, 1.18, and 2.32 mg/m^3 for the light-duty engine and 0.46, 0.96, 1.84, and 3.72 mg/m^3 for the heavy-duty engine. Fischer 344 rats (120 males and 95 females per exposure level for each engine type) were exposed for 16 h/day, 6 days/week for 30 mo. Particle size distributions were determined using an Andersen cascade impactor and an electrical aerosol analyzer. At the 24-mo sampling, the MMAD and distribution (σg) were 0.22 (2.93) and 0.19 (2.71) for the light-duty engine groups at 2.32 and 1.18 mg/m^3, respectively, and 0.27 (3.18) and 0.22 (2.93) for the heavy-duty engine groups at 3.72 and 1.84 mg/m^3, respectively (Ishinishi et al., 1988). The number and timing of the samples are not clear from the published reports, nor is it clear which method was used for the results reported above. Particle size data were not reported for the other exposure groups. Hematology, clinical chemistry, urinalysis, and light and electron microscopic examinations were performed. The body weight of females exposed to 4 mg/m^3 DPM was 15% to 20% less than that of controls throughout the study. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 DPM or less. At concentrations above 0.4 mg/m^3 DPM, accumulation of particle-laden AMs was observed. In areas of AM accumulation, there was bronchiolization of the alveolar ducts, with bronchiolar epithelium replacing alveolar epithelium. Proliferation of brochiolar epithelium and Type II cells was observed. In these areas, edematous thickening and fibrosis of the alveolar septum were seen. Fibrosis of the alveolar septum developed into small fibrotic lesions. These lesions are collectively referred to as hyperplastic lesions by the authors and their incidence is reported. From a total of 123 to 125 animals examined (approximately equal numbers of males and females), hyperplastic lesions were reported in 4, 4, 6, 12, and 87 animals in the light-duty engine groups exposed to 0, 0.11, 0.41, 1.18, and 2.32 mg/m^3 DPM, respectively, and in 1, 3, 7, 14, and 25 animals in the heavy-duty engine groups exposed to 0, 0.46, 0.96, 1.84, and 3.72 mg/m^3 DPM, respectively. Statistical analysis of these results was not reported, but there was no difference in the severity ascribed to changes in pulmonary pathology at similar exposure concentrations between the LD and the HD series.

The ITRI and HERP studies are complementary for identifying the critical effect and its LOAEL and NOAEL. The ITRI study provides results on many different endpoints reflecting pulmonary toxicity, and the effect levels are the same, but the LOAEL and NOAEL are different by a factor of 10. In the HERP study, the concentrations differ by a factor of 2-4, but only

histopathology is reported. Taken together, these two studies (including several published reports for the ITRI study) provide good definition of the low-concentration effects of diesel emissions.

The HERP study identifies LOAELs for rats exposed chronically at 1.18 and 0.96 mg/m^3 (actual exposure) for the LD and HD series, respectively, and NOAELs at 0.41 and 0.46 mg/m^3 (actual) for the LD and HD series. The ITRI studies identify a NOAEL for biochemical, histological, and functional changes in the pulmonary region at 0.35 mg/m^3 (LOAEL = 3.5 mg/m^3). The human equivalent concentrations (HECs) for the principal studies were obtained using the deposition and retention model of Yu and Yoon (1990), as discussed previously. The HEC calculation is based on the assumption that the estimate for the human exposure scenario (a 70-year continuous exposure) should result in an equivalent dose metric, expressed as mass of diesel particle carbon core per unit of pulmonary region surface area, to that associated with no effect at the end of the 2-year rat study. To obtain the HEC, the lung burden in the rat study is calculated using the exposure regimen (concentration, number of hours per day, and days per week) and values for rat tidal volume, functional residual capacity, and breathing frequency. A continuous human exposure resulting in the same final lung burden is calculated and is the HEC. The HEC values corresponding to the animals' exposure levels in the principal studies are shown in Table 6-1, along with a designation of the concentrations as AEL (adverse-effects level) or NOAEL; the LOAELs (HEC) are 0.30, 0.36, and 0.36 mg/m^3. These values, along with the LOAELs from other studies (discussed below), show strong support for an experimental threshold in rats in the range of 0.15 to 0.3 mg/m^3 DPM. The highest NOAEL (HEC), which is below all LOAELs (HEC), is 0.155 mg/m^3 DPM from the HERP heavy-duty diesel study. This NOAEL (HEC) is selected as the basis for the RfC calculation.

6.5. SUPPORTING STUDIES FOR INHALATION REFERENCE CONCENTRATION DERIVATION

Chronic inhalation studies using male F344 rats and male Hartley guinea pigs were carried out at the General Motors (GM) Research Laboratories (Barnhart et al., 1981, 1982). Exposures to target concentrations of 0.25, 0.75, and 1.5 mg/m^3 DPM were generated 20 h/day, 5.5 days/week for up to 2 years. Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo were reported by Barnhart et al. (1981, 1982). The focus of these studies is on electron micrographic morphometry, and very little descriptive light microscopic histology is reported. These data show that no appreciable changes in morphometric parameters occurred after a 2-year exposure to 0.25 mg/m^3, while exposure to 0.75 or 1.5 mg/m^3 DPM resulted in increased thickness of alveolar septa and increased number of various types of alveolar cells. Increased numbers of

Table 6-1. Human equivalent continuous concentrations from the principal studies

Study	Exposure concentration (mg/m^3)	AEL/NOAEL[a]	HEC[b] (mg/m^3)
HERP-Light Duty	0.11	NOAEL	0.038
	0.41	NOAEL	0.139
	1.18	AEL	0.359
	2.32	AEL	0.571
HERP-Heavy Duty	0.46	NOAEL	0.155
	0.96	AEL	0.303
	1.84	AEL	0.493
	3.72	AEL	0.911
ITRI	0.353	NOAEL	0.042
	3.47	AEL	0.360
	7.08	AEL	0.582

[a]AEL: adverse-effects level; NOAEL: no-observed-adverse-effect level.
[b]HEC: human equivalent concentration.

PMNs and monocytes were lavaged from rats exposed to 0.75 or 1.5 mg/m^3, and biochemical changes occurred in lung tissue at these concentrations (Misiorowski et al., 1980; Eskelson et al., 1981; Strom, 1984). These studies demonstrate an LOAEL of 0.796 mg/m^3 DPM and a NOAEL of 0.258 mg/m^3 DPM for male guinea pigs in a chronic study for respiratory endpoints, including light and electron microscopy, lavage cytology, and lung tissue biochemistry.

A 15-mo inhalation study was performed by Southwest Research Institute for General Motors (Kaplan et al., 1983). Male F344 rats, Syrian golden hamsters, and A/J mice were exposed to diluted diesel exhaust at target concentrations of 0.25, 0.75, and 1.5 mg/m^3 for 20 h/day and 7 days/week. Focal accumulation of particle-laden AMs was associated with minimal to mild fibrosis of the alveolar wall. Based on accumulation of particle-laden macrophages, this study identifies an LOAEL at 0.735 mg/m^3 and an NOAEL at 0.242 mg/m^3.

In a study performed by NIOSH (Lewis et al., 1986, 1989; Green et al., 1983), male and female F344 rats and male Cynomolgus monkeys were exposed to target levels of 2 mg/m^3 diesel particles. Accumulations of black-pigmented alveolar macrophages were seen in the alveolar

ducts of rats adjacent to terminal bronchioles, and epithelial lining cells adjacent to collections of pigmented macrophages showed marked Type II cell hyperplasia. No evidence of impaired pulmonary function as a result of the exposure to diesel exhaust was found in rats. Histological examination of lung tissue from monkeys exposed for 24 mo in the same regimen used for rats revealed aggregates of black particles, principally in the distal airways of the lung. Fibrosis, focal emphysema, or inflammation was not observed. The monkeys exposed to diesel exhaust demonstrated small airway obstructive disease. This study demonstrates an LOAEL for rats and monkeys at a diesel particle concentration of 2 mg/m^3. Although the data suggest that the pulmonary function effect in primates more closely resembles that in humans, this study had only one exposed group, making evaluation of dose response impossible. Thus, it was not considered sufficient to eliminate consideration of the strong rodent database.

Heinrich et al. (1986; see also Stöber, 1986) exposed male and female Syrian golden hamsters, female NMRI mice, and female Wistar rats to diesel engine emissions with a 4.2 mg/m^3 particulate concentration. Lung weights were increased by a factor of 2 or 3 in rats and mice after 2 years of exposure, and in hamsters the lung weights were increased by 50% to 70%. Although histological examination revealed different levels of response among the three species, histological effects were seen in all species and effects on pulmonary function were observed in rats and hamsters. This study demonstrates an LOAEL of 4.2 mg/m^3 in rats for respiratory system effects.

The effects of diesel exhaust on the lungs of 18-week-old male Wistar rats exposed to 8.3 ± 2.0 mg/m^3 particulate matter were investigated by Karagianes et al. (1981). Histological examinations of lung tissue noted focal aggregation of particle-laden alveolar macrophages, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema. Lesion severity was related to length of exposure. No exposure-related effects were seen in the nose, larynx, or trachea. This study demonstrates an LOAEL of 8.3 mg/m^3 DPM for respiratory effects after chronic exposure of rats to diesel emissions.

Lung function was studied in adult cats chronically exposed to diesel exhaust concentrations of 6.34 mg/m^3 for the first 61 weeks and 6.7 mg/m^3 from weeks 62 to 124. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks (Pepelko et al., 1980).

Heinrich et al. (1995) exposed Wistar rats to diesel exhaust at DPM concentrations of 0.8, 2.5, and 7 mg/m^3, 18 h/day, 5 days/week for 24 mo. Body weights were significantly decreased in the two higher exposure groups. Bronchoalveolar hyperplasia and interstitial fibrosis of

increasing incidence and severity at greater concentrations were seen in all exposure groups. This study demonstrates an LOAEL of 0.8 mg/m^3.

Nikula et al. (1995) exposed Fischer 344 rats to diesel exhaust at DPM concentrations of 2.4 and 6.3 mg/m^3 16 h/day, 5 days/week for 23 mo. Survival was decreased in the high-exposure males, while body weights were reduced in both males and females in the high-exposure group. Pulmonary hyperplasia, inflammation, and fibrosis were seen in a high percentage of rats in both exposure groups. The high exposure concentrations precluded use of this study for the development of an RfC.

Werchowski et al. (1980a) reported a developmental study in rabbits exposed on days 6 through 18 of gestation to a 1-in-10 dilution of diesel exhaust (DPM concentration ≈ 12 mg/m^3). Exposure to diesel emissions had no effect on maternal toxicity or on the developing fetuses. In a companion study (Werchowski et al., 1980b), 20 SD rats were exposed for 8 h/day during days 5 to 16 to a target concentration of 12 mg/m^3 of DPM. Fetuses were examined for external, internal, and skeletal malformations, and the number of live and dead fetuses, resorptions, implants, corpora lutea, fetal weight, litter weight, sex ratio, and maternal toxicity were recorded. No conclusive evidence of developmental effects was observed in this study.

In an EPA-sponsored reproductive study summarized by Pepelko and Peraino (1983), CD-1 mice were exposed to a target concentration of 12 mg/m^3 DPM for 8 h/day and 7 days/week. The F_0 and F_1 animals were exposed for 100 days prior to breeding, and 100 mating pairs were randomly assigned to four exposure groups of 25 each. Viability counts and pup weights were recorded at 4, 7, and 14 days after birth and at weaning. No treatment-related effects on body weight in F_0 mice or in F_1 animals through weaning or in mating animals through gestation were found. No treatment-related effects on gestation length, percent fertile, litter size, or pup survival were observed. The only organ weight difference was an increase in lung weight in exposed F_0 and F_1 mice (lung weight and lung weight/body weight) and in F_2 males (lung weight/body weight). Based on this study, an NOAEL for reproductive effects in rats is identified at 12 mg/m^3 DPM.

The reproductive and developmental studies described in Chapter 5 show that the effects in the respiratory system are the most sensitive effects that result from diesel exhaust exposures. These studies add to the confidence that a variety of noncancer effects have been studied and are required for a designation of high confidence in the database and the RfC.

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers. The human studies, taken together, are suggestive but inconclusive of an effect on pulmonary function, as described in Chapter 5. The studies are not directly useful for deriving the RfC because of inadequate ability to directly relate the

observed effects to known concentrations of DPM. The studies are confounded by coexposures to other particles or by a lack of measurement of particle exposure.

6.5.1. Respiratory Tract Effects in Species Other Than the Rat

In several of the chronic inhalation studies described in Chapter 5, one or more species other than the rat were also exposed and examined for toxic effects. These should provide a basis for comparison of the effects in rats with the effects in other species. In the study performed at ITRI (Henderson et al., 1988; Mauderly et al., 1988), male and female CD-1 mice were exposed similarly to the rats. The LOAEL and NOAEL in rats and mice from this study would be the same, with the NOAEL for respiratory tract effects being 0.35 mg/m^3 DPM (duration-adjusted NOAEL is 0.074 mg/m^3), although some differences in the severity of the effect were apparent.

In the study conducted by the GM Biomedical Science Department (Barnhart et al., 1981, 1982; Strom, 1984; Gross, 1981), male Hartley guinea pigs as well as F344 rats were chronically exposed to 0.258, 0.796, and 1.53 mg/m^3 DPM. The evidence from this study leads to the conclusion that the LOAEL and NOAEL for rats and guinea pigs are the same, although important differences in the endpoints were reported in the two species. The NOAEL is 0.258 mg/m^3 (duration adjusted NOAEL is 0.17 mg/m^3).

Kaplan et al. (1982) reported a subchronic study in F344 rats, A/J mice, and Syrian golden hamsters exposed to 1.5 mg/m^3 DPM. The histological observations, including AM accumulation and associated thickening of the alveolar wall, were described together, with no distinction between species, suggesting that the observed effects were similar in the species examined. Kaplan et al. (1983) reported a 15-mo study in which F344 rats, A/J mice, and Syrian golden hamsters were exposed to 0.25, 0.75, or 1.5 mg/m^3 DPM. No exposure-related lesions were found in tissues other than the respiratory tract. Based on particle-laden AM accumulation, this study identifies an LOAEL at 0.735 mg/m^3 and an NOAEL at 0.242 mg/m^3. The descriptions provided suggest that the pulmonary effects were similar across the three species examined, but this conclusion is compromised by the lack of detailed reporting and the possibility of intercurrent infection in rats and poor animal health (as evidenced by poor growth) in hamsters. The duration adjusted NOAEL is 0.202 mg/m^3.

Lewis et al. (1986, 1989) exposed rats and monkeys to 2 mg/m^3 DPM for 2 years and reported pulmonary function and histopathology. Pulmonary function was affected in both species, although with a different pattern of response, as discussed in Chapter 5. Significant differences were observed in the histopathological response. In monkeys, slight particle accumulation was observed, but no fibrosis, focal emphysema, or inflammation was present. Rat

lungs in this experiment showed AM accumulation, multifocal histiocytosis, and associated fibrosis and inflammatory cells in the interstitium.

Heinrich et al. (1986) exposed Wistar rats, Syrian golden hamsters, and NMRI mice chronically to 4 mg/m^3 DPM. Lung weight was increased two-fold in mice, 1.5-fold in hamsters, and three-fold in rats. The activity of enzymes recovered in bronchoalveolar lavage was increased to roughly the same extent in rats, mice, and hamsters. Hamsters showed thickened alveolar septa and slight epithelial hyperplasia, with no AM accumulation. Mice also showed epithelial hyperplasia and interstitial fibrosis. Rat lungs had severe inflammatory changes, thickened alveolar septa, hyperplasia, and metaplasia. This study presents the clearest indication of a possibly greater severity in rats compared with other rodent species for noncancer effects. It also suggests that the effect in rats may be qualitatively different, with AM accumulation playing a greater role in pathogenesis in rats than in other rodent species.

Heinrich et al. (1995) also compared effects of chronic diesel exposure on rats and two strains of mice exposed to fairly high concentrations of diesel particles. Similar lung burdens were reported in rats and mice on the basis of particle mass per unit lung wet weight. Lung weight was increased to about the same extent in rats and mice. However, the study is focused on cancer effects, and insufficient information is provided to make a detailed comparison of noncancer histopathology in rats and mice.

Several of the studies described above and in Chapter 7 suggest a significant difference in the carcinogenic response of rats and other experimental animal species. It is less clear whether such a difference holds for noncancer effects at lower exposure levels. The studies described above show similar effect levels for different species for effects that occur earlier or at lower exposure concentration, including accumulation of particles, bronchoalveolar lavage measurements, lung weight, and minor epithelial thickening and hyperplasia. At higher diesel concentrations there are clear differences between rats and the other species tested, especially in the progression to more severe histologically observed endpoints, such as hyperplasia, metaplasia, and inflammatory response. Thus the NOAEL for chronic effects of diesel does not appear to be substantially different among species, although there is some suggestion in the literature of a more sensitive as well as qualitatively different response in rats. This comparison is weakened by the fact that the published reports often give less emphasis to noncancer responses and because the effects in rats and other species are not always measured or reported in the same way. The pathogenesis of diesel exhaust effects has not been studied as thoroughly in any other species as it has in the rat. For example, no specific measurement of particle clearance from the lung has been reported in any species other than the rat. Within the resolving power of the available studies, it is concluded that there is limited evidence for a difference in the NOAEL

for noncancer effects across species, but the evidence is not adequate to quantitatively define the difference, especially at low exposure concentrations. Hence there is no clearly more appropriate species on which the RfC derivation for noncancer effects should be based.

Mice were included in the ITRI, Kaplan et al. (1982), and Heinrich et al. (1986, 1995) studies. The Heinrich studies used a single exposure to high concentrations and are supportive of the other results in mice but are not appropriate to define an LOAEL for mice. The Kaplan study defines an LOAEL and NOAEL of 0.735 and 0.242 mg/m^3 DPM, respectively. The duration-adjusted LOAEL and NOAEL are 0.613 and 0.202 mg/m^3, respectively. The ITRI study defined the adjusted LOAEL and NOAEL at 0.723 and 0.074 mg/m^3, respectively. Because the dose spacing is so wide in the ITRI study, the Kaplan study is more appropriate for defining an NOAEL. Likewise, the Kaplan et al. study is the only multiple-dose study in hamsters, and it defines the same LOAEL and NOAEL for hamsters as for mice. The GM study is the only chronic study in guinea pigs, and it defines the LOAEL and NOAEL for this species at 0.796 and 0.258 mg/m^3, respectively. The adjusted LOAEL and NOAEL for guinea pigs from the GM study are 0.52 and 0.17 mg/m^3, respectively. The effects levels for mice, hamsters, and guinea pigs are similar to the duration-adjusted LOAEL and NOAEL for rats, which are 0.723 mg/m^3 (from ITRI study) and 0.26 mg/m^3 (from Ishinishi et al., 1988), respectively. If the RfC were to be derived based on the duration-adjusted NOAEL, the rat data would be preferred because of the more complete database of chronic rat studies and the more complete presentation of the noncancer endpoints in the rat studies.

The method for deriving inhalation RfCs (U.S. EPA, 1994) includes dosimetric adjustments of animal exposure to arrive at a human equivalent concentration. The default calculation of an HEC for a particle exposure uses the ratio of animal to human regional deposited dose (RDDR) to a specific region of the respiratory tract. The methods also allow replacement of the default approach when a better model is available. The derivation of the RfC in this case makes use of the Yu and Yoon (1990) model to calculate the HEC from the rat studies. Since the Yu and Yoon model has been developed only for the rat-to-human extrapolation, the chosen approach assumes that dosimetric differences between rats and other small animal species would not result in a substantially lower HEC. The LOAEL (HEC) and NOAEL (HEC) from the rat studies based on the Yu and Yoon model are 0.36 and 0.155 mg/m^3, respectively.

6.6. DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

Studies of chronic exposures to diesel emissions performed at ITRI and HERP were selected as the basis of the RfC because they identify both an NOAEL and an LOAEL for rats

exposed chronically. The only other study identifying both an NOAEL and an LOAEL was the GM study, which was not used because information characterizing the pulmonary lesions in rats was limited. The availability of the dosimetric model for rats and not for other species, along with the apparent comparability between the rat and other rodent species in response, resulted in choosing the rat as the basis for developing the RfC. Although the data from the monkey in the Lewis et al. (1989) study suggest that the pulmonary function effect in primates more closely resembles that in humans, this study had only one exposed group, making evaluation of dose response impossible. Thus, this was not considered to be a strong enough basis to eliminate consideration of the strong rodent database. The pulmonary effects, including histological lesions, biochemical changes, pulmonary function impairment, and impaired particle clearance, were determined to be the critical noncancer effect. Sufficient documentation from other studies showed that there is no effect in the extrathoracic region of the respiratory system or in other organs at the lowest levels that produce pulmonary effects in chronic exposures. In addition, adequate information is available from the EPA studies showing no effect on development in two species or on reproduction in a two-generation reproductive study.

Because the RfC is based on an NOAEL from a chronic animal study, uncertainty exists in the extrapolation from animals to humans and for extrapolation to sensitive members of the population (inter- and intraspecies extrapolation). A default factor of 10 is normally applied for each area of uncertainty (i.e., a total uncertainty factor of 100) when a chronic animal NOAEL is available. Since a dosimetry model specifically for diesel particles is available, the use of this model is considered to reduce the uncertainty in extrapolating between animals and humans, compared to a case in which no chemical or species-specific data on dosimetry are available. The default uncertainty factor of 10 includes aspects of pharmacokinetics and pharmacodynamics. An uncertainty factor of 1 rather than 10 was adopted for interspecies extrapolation and was used for the diesel RfC. The uncertainty factor for interspecies extrapolation is normally reduced when kinetic data are available, as in this case, which reduces the uncertainty in extrapolating from animals to humans by accounting for the kinetic differences with data. A further reduction was considered appropriate, as recommended by peer reviewers, on the basis that substantial information suggests that the rat may be a very sensitive species, compared with humans, to the effects of inhalation of diesel particles. There is some evidence, as discussed above, that rats may be more sensitive than other rodent species. There is also evidence from the Lewis et al. (1989) study that rats may be more sensitive than monkeys. Humans would be expected to be more similar to monkeys because their respiratory tract structure is more similar and because of their closer phylogenetic relationship. In the comparison of rat effects with those on other rodents and monkeys, there is also limited evidence that the

responses are qualitatively different, with a much greater role for macrophage dysfunction and accumulation and associated epithelial effects in rats. These lines of evidence, which are fairly limited individually, lead to a reasonably strong argument for a reduction in the uncertainty factor when considered together. A total uncertainty factor of 10 results for intraspecies extrapolation.

With the NOAEL (HEC) of 0.155 mg/m^3 DPM from the HERP study, an RfC of 16 μg/m^3 was calculated. The RfC also includes confidence statements associated with the principal study, the database, and the resulting RfC. The studies used as the basis for the RfC were well-conducted chronic studies with adequate numbers of animals, and the LOAELs and NOAELs were consistent across studies, thereby resulting in high confidence. The database contains several chronic studies, including multiple species, that support the LOAEL observed in the principal studies. There are also developmental and reproductive studies, resulting in a high-confidence data base. Because of the high confidence in the studies and database, the RfC has high confidence.

6.6.1. Application of the Benchmark Dose Approach to Derivation of the RfC

An alternative to deriving the RfC based on the NOAEL identified in the animal studies is application of the benchmark dose/concentration approach. The BMC was described by Crump (1984) and recently discussed by EPA (1995b). The BMC approach involves fitting a dose-response function to dose and effect information from a single study and using the dose-response curve to predict the dose that will result in a level of response that is defined a priori as the benchmark response. For example, a 10% increase in incidence of epithelial hyperplasia might be defined as the benchmark response, and a dose-response curve relating inhaled DPM to hyperplasia in rats exposed chronically to diesel exhaust would be used to estimate the exposure concentration resulting in a 10% increase. The lower confidence limit of that concentration is the BMC, and it is used as the representative value for the dose-response assessment.

For diesel exhaust there are several chronic exposure studies in animals that could be used to estimate a benchmark concentration. Software for performing benchmark dose calculations is available commercially, and benchmark dose programs also can be developed using standard statistical software. Different approaches are used for modeling dichotomous versus continuous data. Dichotomous models estimate the probability of the effect being modeled at a given dose, and continuous models estimate the magnitude of the response at a given dose. This fundamentally different output causes difficulty in cases such as the database for diesel exhaust that include both types of data, because comparisons of the two types of results must be made to select the most appropriate BMC for the dose-response assessment. Crump

(1995) has developed an approach to modeling continuous data that models dose against the distribution of the continuous variable and estimates the probability of an abnormal response at a given dose using a preselected magnitude of response to define an abnormal response. This approach has the distinct advantage of expressing the results for dichotomous and continuous data in the same terms, but it has not been evaluated extensively and is not readily available.

A first step in carrying out a BMC analysis is selecting studies and data sets that are appropriate to model. Minimum data criteria have not been clearly established. As provisional criteria, the analyses that follow will require that complete information on the response of interest should be available and that at least two exposure levels with responses that differ from those of the controls are needed. Based on this criterion, studies with a single exposure concentration (including Lewis et al., 1989; Heinrich et al., 1986; Iwai et al., 1986; Karagianes et al., 1981; Pepelko et al., 1980; and the mouse data from Heinrich et al., 1995) are not amenable to the BMC approach. In addition, the rat data from Heinrich et al. (1995) and Creutzenberg et al. (1990) are not amenable to BMC analysis because the information on noncancer histopathology is not reported in detail and the information on lung clearance rates is reported as group means with no standard deviation.

To perform a dose-response analysis for a continuous variable, either a measure of variability or the individual animal measurements are needed. The study reported by Kaplan et al. (1983) is also not considered useful for BMC analysis. The principal result of interest in the Kaplan study is reported as the incidence of pneumoconiosis in rats, mice, and hamsters. The term pneumoconiosis appears to be applied to the presence of DPM in the lung and in AMs as well as AM accumulation and any secondary effects in the epithelium. Epithelial effects in the area near particle-laden AMs are discussed in the text of the Kaplan study but are not listed as a separate entity in the tables of effects. Thus the effect termed pneumoconiosis appears to include both the expected dose-related increase in particles in the lung and any adverse effects occurring secondary to particle deposition, and therefore does not represent a clearly adverse effect. The existence of some studies for which the BMC approach is feasible and some for which it is not feasible introduces a potentially serious difficulty in deciding on the most appropriate dose-response value to use in deriving the RfC. In the case of diesel exhaust, however, this concern is reduced because the studies identified as the most appropriate for RfC derivation based on NOAEL/LOAEL levels are also amenable to BMC analysis.

The studies for which BMC analyses were performed were the ITRI, General Motors, Ishinishi (1986), and Nikula et al. (1995) studies. These studies contained a variety of endpoints that could be modeled using the BMC approach. A total of 41 data sets were selected from these studies for BMC determination. Because a variety of models are available, and each model can

be varied (for example, by including a background term or a threshold term), the number of model runs for a database as complete as that for diesel exhaust can become unmanageable. In addition to this large number of endpoints and models, several dose or exposure terms could be used (e.g., for rats one could use the exposure concentration, the exposure concentration × time, the duration-average exposure concentration, the lung burden estimated by a deposition model, or the human equivalent concentration based on a deposition and clearance model). Clearly, the number of possible model runs expands geometrically, and some decisions must be made to limit and focus the extent of the analyses.

In these analyses, the dose metric used for rat data was the human equivalent continuous exposure concentration based on the Yu and Yoon (1990) deposition/clearance model. For mice, the duration-averaged exposure concentration was used. The 41 data sets were modeled using the polynomial model dichotomous or continuous data (Howe, 1990a, 1990b), based on extra risk, and including or excluding a threshold term. The inclusion of a threshold term improved model fit substantially only in cases with low-dose groups showing no response over controls. In most cases a background term was also estimated, and in some analyses with dichotomous data it was omitted. As expected, the inclusion of a background term improved model fit for dichotomous data only when there was a nonzero response in the controls. Based on the results using the polynomial model, 12 data sets were selected for analysis with the Weibull model (Howe, 1990c, 1990d) to determine the sensitivity of the BMC estimate to the choice of model. The availability of different dose metrics and different models for different endpoints introduces additional difficulty in determining the most appropriate BMC for deriving the RfC. This difficulty is lessened by the fact that the rat data for BMC analysis are the most extensive, they include several studies for which both LOAEL and NOAEL are identified, and they are also the data for which the deposition and clearance model is available. As discussed previously, the data from other species do not suggest large differences in species sensitivity at low concentrations.

Perhaps the most critical decision in the BMC approach is the level of response defined as the benchmark response. As discussed by EPA (1995b), there is an emerging consensus that a BMR of 0.05 or 0.1 is probably appropriate for dichotomous data for most endpoints. There is no clear consensus about the appropriate choice of BMR or on how to select a BMR for a continuous effect. This dilemma is the main reason for the appeal of models such as that presented by Crump (1995) or by Gaylor and Slikker (1990), which derive a BMC in terms of a probability statement. However, both of those models require that some magnitude of response be selected to delineate between "responders" and "nonresponders," so the issue remains as to how one can consistently define a response level for the myriad endpoints found in the toxicological literature. One approach is to work backward from the way the BMC will be used

to derive the RfC. The discussion by EPA (1995b) and the precedents for the use of BMC in derivation of RfCs that are now on IRIS suggest that the BMC will be used like an NOAEL has been used in the past. It follows that the BMR should be set at a level that would not be considered adverse for the effect in question, or for an effect that is very mildly adverse such that the use of the lower confidence limit on dose results in a BMC that is in the nonadverse range under the conditions of the experiment. This provides general guidance for selecting BMRs for continuous endpoints, although the issue of maintaining consistency between endpoints remains an extremely important one because of the interrelatedness of different toxicological endpoints. The benchmark responses used in the BMC analyses for diesel exhaust are shown in Table 6-2.

It should be noted that the lack of clear policy on selecting the BMC and the lack of specific guidance for comparing different respiratory tract effects in the form of target organ-specific guidelines, along with the other issues raised above, make BMC analysis a possible but not automatic alternative to the derivation of the RfC as presented. The BMC analysis for diesel is presented here for comparison with the RfC based on the LOAEL/NOAEL from the rat studies as presented above.

In the ITRI study, in particular, many endpoints were amenable to benchmark modeling. Henderson et al. (1988) present data on lung weight and lavage biochemistry and cytology from rats and mice sacrificed at various time points during a chronic study. Wolff et al. (1987) present data on particle clearance half-times in rats. The results shown in Table 6-3 are based on the best-fitting exponential polynomial model using the BMR from Table 6-2 applied to the ITRI data (Henderson et al., 1988; Wolff et al., 1987).

Table 6-2. Definition of benchmark response levels for endpoints important in the diesel exhaust BMC analysis

Endpoint	Benchmark definition	Extra risk
Lung weight	10% increase	0.10
Bronchoalveolar lavage-number of macrophages	50% increase	0.50
Bronchoalveolar lavage-number of neutrophils	200% increase (3 × control)	2.0
Bronchoalveolar lavage-protein	100% increase (2 × control)	1.0
Bronchoalveolar lavage-enzymes	100% increase (2 × control)	1.0
Clearance half-time	20% increase	0.20
Body burden of a particle after 200 days	20% increase	0.20
Incidence of hyperplasia	10% incidence	0.10
Alveolar-capillary thickness	20% increase	0.20

Table 6-3. Results of benchmark concentration analyses using the polynomial model and data from the ITRI study

Data set, model	Benchmark response	MLE	BMC
Rats			
Male rat lung weight @ 24 mo Polynomial, no threshold	10	0.10	0.08
Female rat lung weight @ 24 mo Polynomial, no threshold	10	0.061	0.05
Rat BAL macrophages @ 24 mo, M and F combined Polynomial, no threshold	50	0.315	0.27
Rat BAL neutrophils @ 24 mo, M and F combined Polynomial, threshold	200	0.0942	0.06
Rat BAL protein @ 18 mo, M and F combined Polynomial, threshold	100	0.309	0.18
Rat BAL LDH @ 18 mo, M and F combined Polynomial, no threshold	100	0.221	0.15
Rat BAL β-glucuronidase @ 24 mo, M and F combined Polynomial, threshold	100	0.172	0.05
Rat Ga_2O_3 clearance half-time @ 6 mo, M and F combined Polynomial, no threshold	20	0.262	0.15
Rat Ga_2O_3 clearance half-time @ 12 mo, M and F combined Polynomial, no threshold	20	0.168	0.10
Rat Ga_2O_3 clearance half-time @ 24 mo, M and F combined Polynomial, no threshold	20	0.074	0.04
Rat Cs-FAP clearance half-time @ 24 mo, M and F combined Polynomial, no threshold	20	0.048	0.04
Rat Cs-FAP clearance, % of initial body burden after 200 days Polynomial, no threshold	20	0.044	0.03
Mice			
Male mouse lung weight @ 24 mo Polynomial, no threshold	10	0.13	0.11
Female mouse lung weight @ 24 mo Polynomial, no threshold	10	0.16	0.13
Mouse BAL macrophage @ 24 mo, M and F combined Polynomial, no threshold	10	Not able to converge	
Mouse BAL neutrophils @ 24 mo, M and F combined Polynomial, no threshold	200	0.640	0.47
Mouse BAL protein @ 18 mo, M and F combined Polynomial, no threshold	100	0.353	0.27
Mouse BAL LDH @ 18 mo, M and F combined Polynomial, no threshold	100	0.455	0.36
Mouse BAL β-glucuronidase @ 24 mo, M and F combined Polynomial, threshold	100	0.112	0.11

Source: Henderson et al. (1988) and Wolff et al. (1987).

The BMC values are the lower 95% confidence limit on the exposure concentration predicted to result in the BMR response level. All of the responses modeled above were data presented as continuous variables, and the model was used to estimate the exposure predicted to result in a predefined response magnitude, the BMR. Without any other adjustment for the severity of the effect, it is implicit in the BMC approach for continuous data that BMRs are assumed to represent effects of equivalent severity. Clearly, such a comparison is very subjective and cannot be made precisely. Nevertheless, the judgment of the appropriate BMR is fundamental to the application of the BMC approach.

The BMCs from the ITRI rat data were calculated using the human equivalent concentration of the rat exposures based on the Yu and Yoon (1990) dosimetry model. The NOAEL from the ITRI study was 0.35 mg/m^3 (duration-adjusted NOAEL is 0.074 mg/m^3) and the human equivalent NOAEL is 0.042 mg/m^3. This NOAEL (HEC) is lower than the NOAEL from the Ishinishi et al. study, which was used to derive the RfC because the NOAEL and LOAEL from the ITRI study differed by a factor of 10. The rat NOAEL used to derive the RfC was a NOAEL (HEC) of 0.155 mg/m^3. The BMC values based on rat lung weight were below the NOAEL from Ishinishi et al. (1986), suggesting that the lung weight is a sensitive effect. The sensitivity of the lung weight is consistent with other chronic rat studies as well as those in mice (Heinrich et al., 1995). The bronchoalveolar lavage indicators showed large variability in BMC value, with macrophages, protein, and LDH having BMCs above the range of the rat NOAEL. Lavagable neutrophils and β-glucuronidase, however, were quite sensitive. Measures of particle clearance in rat lungs were also sensitive, resulting in a range of BMC values between 0.033 and 0.146 mg/m^3. It was noted that there are no BMC estimates based on histopathological effects, even though these effects tend to be sensitive indicators of diesel exposure, because these effects were not reported in an adequately quantitative manner in the various publications describing the ITRI study.

The BMC levels based on mouse data from the ITRI study also showed a wide range, with lung weight and lavage β-glucuronidase being the most sensitive. The mouse BMCs were calculated using the duration-averaged exposure concentration as the dose term, so the appropriate NOAEL for comparison is the duration-adjusted NOAEL from the ITRI study, which was 0.074 mg/m^3. The duration-averaged LOAEL for the same study is 0.723 mg/m^3. All mouse BMC values from the ITRI study fall between the NOAEL (HEC) and the LOAEL (HEC).

The basis for the RfC was the NOAEL (HEC) in the Ishinishi study, which was 0.155 mg/m^3. This NOAEL (HEC) was selected because it was lower than the valid LOAELs in rats from all studies considered and higher than the other NOAEL (HEC) values. The only data in

the Ishinishi et al. (1988) study that were amenable to BMC analysis were the incidence of hyperplastic lesions in male, female, and combined. These results are shown in Table 6-4.

The BMC for the most sensitive effect is very similar to the NOAEL (HEC). Most of the BMC values fall between the LOAEL (HEC) and the NOAEL (HEC) from the same data set. Two of the BMCs for the heavy-duty diesel experiment exceed the LOAEL (HEC). This could result from the large number of animals and close dose spacing, which allows identification of a lower LOAEL (HEC). These data sets were characterized by very low incidence of lesions at the lowest exposure level, and the assignment of the LOAEL (HEC) was difficult because of the low incidence and the lack of detailed description of the extent or severity of the response. The BMC procedure makes determination of the effect level more objective for these data.

Two other studies contained information that was amenable to the BMC approach: the chronic study done at GM and the Nikula et al. (1995) study. Results of these analyses are shown in Table 6-5.

Table 6-4. Results of benchmark concentration analyses using the polynomial model and data from the HERP study

Data set, model	Benchmark response	MLE[a]	BMC[a]
HERP light-duty diesel, hyperplastic lesions, male and female combined Polynomial, threshold, background	10	0.39	0.34
HERP light-duty diesel, hyperplastic lesions, male rats Polynomial, no threshold, background	10	0.35	0.32
HERP light-duty diesel, hyperplastic lesions, female rats Polynomial, no threshold	10	0.24	0.19
HERP heavy-duty diesel, hyperplastic lesions, male and female combined Polynomial, no threshold	10	0.49	0.38
HERP heavy-duty diesel, hyperplastic lesions, male rats Polynomial, no threshold	10	0.67	0.46
HERP heavy-duty diesel, hyperplastic lesions, female rats Polynomial, no threshold	10	0.43	0.30

[a]mg/m^3.
Source: Ishinishi et al. (1988).

Table 6-5. Results of benchmark concentration analyses using the polynomial model and data from the GM and Nikula studies

Data set, model	Benchmark response	MLE[a]	BMC[a]
GM study—continuous variables			
Male rat alveolar-capillary thickness @ 6 mo Polynomial, threshold	20	0.069	0.06
Male rat BAL PMNs @ 48 weeks Polynomial, no threshold	200	0.087	0.08
Male rat BAL macrophages @ 48 weeks Polynomial, no threshold	50	0.244	0.12
Nikula study—continuous variables			
Male RT Lu wt. @ 23 mo Polynomial, no threshold	10	0.088	0.06
Female RT Lu wt. @ 23 mo Polynomial, no threshold	10	0.039	0.03
Male RT Lu wt. @ 18 mo Polynomial, no threshold	10	0.170	0.13
Female RT Lu wt. @ 18 mo Polynomial, no threshold	10	0.122	0.04
Nikula study—dichotomous variables			
Male RT chronic inflammation @ >18 mo Polynomial, no threshold	10	0.232	0.14
Female RT chronic inflammation @ >18 mo Polynomial, no threshold	10	0.095	0.08
Female RT alveolar proteinosis @ >18 mo Polynomial, no threshold	10	0.122	0.03
Male RT bronchoalveolar metaplasia @ >18 mo Polynomial, no threshold	10	0.195	0.12
Female RT bronchoalveolar metaplasia @ >18 mo Polynomial, no threshold	10	0.026	0.02
Male RT focal fibrosis and epithelial hyperplasia @ >18 mo Polynomial, no threshold	10	0.721	0.46
Female RT focal fibrosis and epithelial hyperplasia @ >18 mo Polynomial, no threshold	10	0.336	0.20

[a]mg/m^3.
Source: Barnhart et al. (1981, 1982) and Nikula (1995).

Overall, the benchmark concentrations from several experiments and a variety of endpoints support the NOAEL and LOAEL identified from the rat database for chronic diesel exposure studies. It is common practice to apply several models to the key data sets in a benchmark concentration analysis to determine whether the BMC is significantly model dependent, and if so, to select the most appropriate model. One reason for using a BMR that is close to the observable range is that the various models used for dose-response analysis tend to diverge more as they are extrapolated to lower doses and at the BMR such extrapolation should not be necessary, so substantial model dependence of the result is less likely. Several of the data sets that resulted in lower BMC estimates were modeled using the Weibull model, and the results are shown in Table 6-6.

These results indicate that there is relatively little difference among models for a variety of studies and endpoints. The BMC analyses shown in Table 6-6 were selected because they resulted in the lowest BMC levels of the studies and endpoints evaluated. It has been suggested that the lowest BMC level should be used to derive the RfC, analogous to the use of the lowest LOAEL to derive the RfC. Selection of the most appropriate BMC to derive the RfC is complicated by the fact that there may be many BMCs from a given experiment that identifies a single LOAEL and NOAEL. The ITRI study, for example, identifies an LOAEL of 3.5 mg/m^3 and an NOAEL of 0.35 mg/m^3 (exposure concentrations), but because of the large number of endpoints studied, many BMC values are available. On the other hand, a study such as Ishinishi et al. (1986) has only one noncancer endpoint that can be used for BMC analysis, and the Heinrich et al. (1995) study had no noncancer endpoints that were presented in a sufficiently quantitative manner for BMC analysis. If these studies are not considered in an analysis because BMC models cannot be applied, substantial uncertainty would be introduced because significant information is being ignored. In addition, several caveats must be considered relating to the studies for which BMCs were calculated. The endpoints of chronic inflammation and alveolar proteinosis in the female rats in the Nikula study were based on BMC models fit to two exposure groups, and the incidences in the lowest exposed group were 49% and 83%, respectively. Thus, the BMC is estimated at a concentration well below the lowest data point, and this extrapolation may be cause for concern. This concern applies equally to the lung weight data in the Nikula study, which show a greater than twofold increase in the low-concentration females. The data on pulmonary particle clearance from the ITRI study were reported as an estimate of the clearance half-time with no measure of variability. To run the BMC model, a coefficient of variability of 20% was assumed. Thus the data themselves do not meet the minimum requirements for application of the BMC approach as discussed above. These data sets result in the lowest BMC levels in the diesel database. For several other endpoints, such as alveolar-capillary thickness

Table 6-6. Comparison of benchmark concentration calculated using the polynomial and Weibull models for selected endpoints

Data set, model	Benchmark response	MLE[a]	BMC[a]
Dichotomous variables			
HERP light-duty diesel, hyperplastic lesions, female rats			
Polynomial, no threshold	10	0.247	0.19
Weibull, no threshold	10	0.350	0.29
HERP heavy-duty diesel, hyperplastic lesions, female rats			
Polynomial, no threshold	10	0.425	0.30
Weibull, no threshold	10	0.273	0.17
Nikula study, female RT chronic inflammation @ >18 mo			
Polynomial, no threshold	10	0.095	0.08
Weibull, no threshold	10	0.102	0.09
Nikula study, female RT alveolar proteinosis @ >18 mo			
Polynomial, no threshold	10	0.122	0.03
Weibull, no threshold	10	0.175	0.03
Continuous variables			
ITRI rat Ga_2O_3 clearance half-time @ 24 mo, M and F combined			
Polynomial, no threshold	20	0.074	0.04
Weibull, threshold	20	0.176	0.07
ITRI rat Cs-FAP clearance half-time @ 24 mo, M and F combined			
Polynomial, no threshold	20	0.048	0.03
Weibull, no threshold	20	0.050	0.03
ITRI male rat lung weight @ 24 mo			
Polynomial, no threshold	10	0.103	0.08
Weibull, no threshold	10	0.103	0.08
ITRI female rat lung weight @ 24 mo			
Polynomial, no threshold	10	0.061	0.05
Weibull, no threshold	10	0.068	0.05
ITRI rat BAL neutrophils @ 24 mo, M and F combined			
Polynomial, threshold	200	0.094	0.06
Weibull, no threshold	200	0.094	0.06
ITRI rat BAL β-glucuronidase @ 24 mo, M and F combined			
Polynomial, threshold	100	0.172	0.05
Weibull, threshold	100	0.176	0.07
G.M. study, male rat alveolar-capillary thickness @ 6 mo			
Polynomial, threshold	20	0.069	0.06
Weibull, no threshold	20	0.069	0.06
G.M. study, male rat BAL PMNs @ 48 weeks			
Polynomial, no threshold	200	0.087	0.08
Weibull, no threshold	200	0.087	0.09

[a] mg/m^3.

and lavage β-glucuronidase level, it is not clear that the BMR selected (20% and 100% increase, respectively) is the appropriate level in the context of applying the BMC to derive the RfC. Despite these caveats, it is clear that the BMC values for the more sensitive endpoints tend toward concentrations that are at or below the NOAEL identified by the combined rat database. This may well result in part because the ITRI study, which is the best documented study, has a tenfold difference between the LOAEL and NOAEL, and the Ishinishi study from which the rat NOAEL was obtained uses much closer dose spacing (a factor of 2.5 between LOAEL and NOAEL) and reports in much less detail on the noncancer effects. Thus the lack of detailed investigation in the Ishinishi study might allow a higher NOAEL to be identified at a level only about one-half of the LOAELs from the ITRI, GM, Lewis et al. (1989), and Heinrich (1995) studies. In other words, the BMC analyses suggest that a lower rat NOAEL might have been identified if different dose spacing and more detailed investigation of noncancer endpoints had been used in the existing studies.

Several limitations have been mentioned in the preceding text regarding the use of the BMC analysis for deriving an RfC. The principal limitations are the following:

- Some key studies in rats have inadequate quantitative data for BMC.
- Some endpoints are amenable to BMC and others are not.
- The policy for selecting BMC from many endpoints is not yet clear.
- It is not clear how to compare dichotomous and continuous BMCs.
- There is a lack of precedent or guidance for selecting BMR levels.
- A deposition model is available only for rats (it is not clear how to compare BMCs based on deposition/retention models with BMC based on default duration-adjusted concentrations).

Because of the issues and questions raised by these aspects of the BMC approach, the BMC will not be used to derive the RfC at this time.

6.7. SUMMARY

A large number of studies of chronic DPM inhalation in laboratory animals are available. These studies characterize the respiratory effects and the concentration-response relationship of those effects in detail. Many epidemiologic studies of occupationally exposed humans also are available. The epidemiologic studies provide qualitative evidence that supports the identification of a hazard to the respiratory system from laboratory animal studies. The human studies are of limited value quantitatively because of their inadequate exposure characterization and confounding by concurrent exposure to other pollutants. The laboratory animal studies are used to derive an RfC. The chronic studies from ITRI and HERP were selected as the principal

studies for RfC development. The deposition and retention model discussed in Chapter 4 and Appendix B was used to calculate human equivalent concentrations and identified a NOAEL (HEC) of 0.155 mg/m^3 from the HERP studies and a LOAEL(HEC) of 0.36 mg/m^3 from the ITRI studies. An uncertainty factor of 10 was applied to account for sensitive members of the population, resulting in an RfC of 16 $\mu g/m^3$. The RfC is considered to have high confidence attributable to high confidence in the study and database.

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7. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

7.1. INTRODUCTION

The particulate phase of diesel exhaust is composed of aggregates of carbon particles; the primary particle diameter ranges from 10 to 80 nm, and aggregates of these primary particles have mass median diameters averaging 0.2 to 0.3 μm (Vuk et al., 1976; Carpenter and Johnson, 1979), although some may approach 1.0 μm. A variety of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), are adsorbed to this carbon.core (see Tables 2-8 and 2-10) and comprise 5% to 65% of the total particle mass (Cuddihy et al., 1984). Some of these organic compounds, such as benzo[a]pyrene (B[a]P), dinitropyrenes, and 1-nitropyrene, have received special attention regarding their carcinogenic and mutagenic potential. 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 composition, engine design, and engine operating conditions. Although less emphasis has been placed on the gaseous phase, potential carcinogens such as formaldehyde, acetaldehyde, benzene, as well as lower molecular weight PAHs, may also be present in this fraction.

The respirability of these particles and their associated organics provides a basis for health hazard concerns, and the reported mutagenicity (Huisingh et al., 1978) and skin papilloma induction (Kotin et al., 1955) of solvent extracts of diesel particulate matter (DPM) suggests a potential for carcinogenicity. Zamora et al. (1983) provided evidence that DPM extracts contained components that acted as weak tumor promoters in vitro. Recently, emphasis has been directed toward assessing the carcinogenic potential of whole and filtered diesel exhaust using whole-animal studies and understanding the mechanisms and implications of deposition, retention, and clearance of the particulate phase of diesel exhaust.

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. Experimental protocols for the inhalation studies usually consisted of exposure (usually chronic) to diluted exhaust in whole-body exposure chambers using rats, mice, and hamsters as 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 DPM and its adsorbed components. Inhalation exposure to DPM alone, however, was not reported. Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 mg/m^3. Clean air (usually filtered) was used in the control exposures. Studies providing both positive, negative, or inconclusive findings have been reported. In this chapter, any indication of statistical significance implies that $p \leq 0.05$ was

reported in the reviewed publications. The experimental protocols and exposure atmosphere characterizations are not described in detail here but may be found in Appendix A. A summary of the animal carcinogenicity studies and their results is presented in Table 7-1.

Also included are studies that assessed the carcinogenic and tumorigenic effects of DPM and solvent extracts of these particles following dermal application, subcutaneous (s.c.) injection, intraperitoneal (i.p.) injection, or intratracheal (itr.) instillation in rodents, as well as co-carcinogenicity studies. Individual chemicals present in the gaseous phase or adsorbed to the particle surface were not included in this review because adequate assessments of those of likely concern (i.e., formaldehyde, acetaldehyde, benzene, PAHs) have been published in other health assessment documents.

7.2. INHALATION STUDIES

7.2.1. Rat Studies

Mauderly et al. (1987) provided data affirming the carcinogenicity of automotive diesel engine exhaust in F344/Crl rats following chronic inhalation exposure. Male and female rats were exposed to diesel engine exhaust at nominal DPM concentrations of 0.35 (n = 366), 3.5 (n = 367), or 7.1 (n = 364) mg/m^3 for 7 h/day, 5 days/week for up to 30 mo. Sham-exposed (n = 365) controls breathed filtered room air. A total of 230, 223, 221, and 227 of these rats (sham-exposed, low-, medium-, and high-exposure groups, respectively) were examined for lung tumors. These numbers include those animals that died or were euthanized during exposure and those that were terminated following 30 mo of exposure. 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 midsize passenger car. The D-2 diesel control fuel (Phillips Chemical Co.) met U.S. EPA certification standards and contained approximately 30% aromatic hydrocarbons and 0.3% 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. Mokler et al. (1984) provided a detailed description of the exposure system. The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A. No exposure-related changes in body weight or life span were noted for any of the exposed animals nor were there any signs of overt toxicity. Collective lung tumor incidence was greater (z statistic, $p \leq 0.05$) in the high (7.1 mg/m^3) and medium (3.5 mg/m^3) exposure groups (12.8% and 3.6%, respectively) versus the control and low (0.35 mg/m^3) exposure groups (0.9% and 1.3%,

Table 7-1. Summary of animal carcinogenicity studies

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenocarcinoma + squamous cell carcinomas	Squamous cysts	All tumors	
Mauderly et al. (1987)	Rat/F344	M + F, 230[b]	Clean air	0	None	7 h/day,	NA	(0)	(0.9)	(0)	(0.9)	
		M + F, 223	Whole exhaust	0.35	None	5 days/week		(0)	(1.3)	(0)	(1.3)	
		M + F, 221	Whole exhaust	3.5	None	up to 30 mo		(2.3)	(0.5)	(0.9)	(3.6)[c]	
		M + F, 227	Whole exhaust	7.1	None			(0.4)	(7.5)	(4.9)	(12.8)[c]	
								Adenomas	Adenocarcinomas	Squamous cell carcinoma	Adeno-squamous carcinoma	Other neoplasms
Nikula et al. (1995)	Rat/F344	M + F, 214[b]	Clean air	0	None	16 h/day,	6 weeks	1/214 (<1)	1/214 (<1)	1/214 (<1)	0/214 (0)	0/214 (0)
		M + F, 210	Whole exhaust	2.5	None	5 days/week		7/210 (3)	4/210 (2)	3/210 (1)	0/210 (0)	0/210 (0)
		M + F, 212	Whole exhaust	6.5	None	for up to		23/212 (11)	22/212 (10)	3/212 (1)	1/212 (<1)	0/212 (0)
		M + F, 213	Carbon black	2.5	None	24 mo		3/213 (1)	7/213 (3)	0/213 (0)	0/213 (0)	1/213 (<1)
		M + F, 211	Carbon black	6.5	None			13/211 (6)	21/211 (10)	3/211 (1)	2/211 (<1)	0/211 (0)
								Adenomas	Carcinomas	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b)	Rat/ Wistar	F, 96	Clean air	0	None	19 h/day,	NA	0/96 (0)	0/96 (0)	0/96 (0)	0/96 (0)	
		F, 92	Filtered exhaust	0	None	5 days/week for up to		0/92 (0)	0/92 (0)	0/92 (0)	0/92 (0)	
Mohr et al. (1986)		F, 95	Whole exhaust	4.0	None	35 mo		8/95 (8.4)	0/95 (0)	9/95 (9.4)	17/95 (17.8)[c]	
								Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b)	Mouse/ NMRI	M + F, 84	Clean air	0	None	19 h/day,	NA	9/84 (11)	2/84 (2)	—	11/84 (13)	
		M + F, 93	Filtered exhaust	0	None	5 days/week for up to		11/93 (12)	18/93 (19)[c]	—	29/93 (31)[c]	
		M + F, 76	Whole exhaust	4.0	None	30 mo		11/76 (15)	13/76 (17)[c]	—	24/76 (32)[c]	
	Hamsters /Syrian	M + F, 96	Clean air	0	None	19 h/day,	NA	0/96 (0)	0/96 (0)	0/96	0/96 (0)	
		M + F, 96	Filtered exhaust	0	None	5 days/week for up to		0/96 (0)	0/96 (0)	0/96	0/96 (0)	
		M + F, 96	Whole exhaust	4.0	None	30 mo		0/96 (0)	0/96 (0)	0/96	0/96 (0)	

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]				Comments
										Squamous cell carcinoma	All lung tumors	
Henrich et al. (1989a)	Rat/ Wistar	F, NS	Clean air	0	DPN[d]	19 h/day, 5 days/week for 24 to 30 mo	NA			(4.4)	(84.8)	
		F, NS	Whole exhaust	4.2	DPN[d]					(46.8)[c]	(83.0)	
		F, NS	Filtered exhaust	0	DPN[d]					(4.4)	(67.4)	
		F, NS	Clean air	0	DPN[e]					(16.7)	(93.8)	
		F, NS	Whole exhaust	4.2	DPN[e]					(31.3)[c]	(89.6)	
		F, NS	Filtered exhaust	0	DPN[e]					(14.6)	(89.6)	
								Adenomas	Adenocarcinomas	Squamous cell carcinomas	Benign squamous cell tumors	
Heinrich et al. (1995)	Rat/ Wistar	F, 220	Clean air	0	None	18 h/day, 5 days/week, for up to 24 mo	6 mo	0/217 (0)	1/217 (<1)	0/217 (0)	0/217 (0)	
		F, 200	Whole exhaust	0.8	None			0/198 (0)	0/198 (0)	0/198 (0)	0/198 (0)	
		F, 200	Whole exhaust	2.5	None			2/200 (1)	1/200 (<1)	0/200 (0)	7/200 (3.5)	
		F, 100	Whole exhaust	7.0	None			4/100 (4)	4/100 (4)	2/100 (2)	14/100 (14)	Tumor incidences after 30 mo
		F, 100	Carbon black	11.6	None			13/100 (13)	13/100 (13)	4/100 (4)	20/100 (20)	
		F, 100	TiO_2	10.0	None			4/100 (4)	13/100 (13)	3/100 (3)	20/100 (20)	
	Mouse/ C57BL/ 6N	F, 120	Clean air	0	None	18 h/day, 5 days/week, for up to 21 mo	6 mo					5.1% tumor rate
		F, 120	Whole exhaust	4.5	None							8.5% tumor rate
		F, 120	Particle-free exhaust	0	None							3.5% tumor rate
	Mouse/ NMRI	F, 120	Clean air	0	None	18 h/day, 5 days/week for up to 13.5 mo	9.5 mo	(25)	(15.4)			
		F, 120	Whole exhaust	4.5	None			(21.8)	(15.4)			
			Carbon black	11.6	None			(11.3)	(10)			
			TiO_2	10	None			(11.3)	(2.5)			
	Mouse/ NMRI	F,120	Clean air	0	None	18 h/day, 5 days/week, 23 mo	None	(25)	(8.8)			
		F,120	Whole exhaust	4.5	None			(18.3)	(5.0)			
		F,120	Particle-free exhaust	0	None			(31.7)	(15)			

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenosquamous carcinomas	Squamous cell carcinomas	All tumors	
Takaki et al. (1989) Light-duty engine	Rat/F344	M + F, 123	Clean air	0	None	16 h/day, 6 days/week, for up to 30 mo	NA	1/23 (0.8)	2/123 (1.6)	1/23 (0.8)	4/123 (3.3)	
		M + F, 123	Whole exhaust	0.1	None			1/23 (0.8)	1/23 (0.8)	1/23 (0.8)	3/123 (2.4)	
		M + F, 125	Whole exhaust	0.4	None			1/25 (0.8)	0/125 (0)	0/125 (0)	1/125 (0.8)	
		M + F, 123	Whole exhaust	1.1	None			0/23 (0)	5/123 (4.1)	0/123 (0)	5/123 (4.1)	
		M + F, 124	Whole exhaust	2.3	None			1/24 (8.1)	2/124 (1.6)	0/124 (0)	3/124 (2.4)	
								Adenomas	Adenosquamous carcinomas	Squamous cell carcinomas	All tumors	
Ishinishi et al. (1988a) Heavy-duty engine	Rat/F344	M + F, 123	Clean air	0	None	16 h/day, 6 days/week, for up to 30 mo	NA	0/123 (0)	1/123 (0.8)	0/123 (0)	1/123 (0.8)	
		M + F, 123	Whole exhaust	0.5	None			0/123 (0)	0/123 (0)	1/123 (0.8)	1/123 (0.8)	
		M + F, 125	Whole exhaust	1.0	None			0/125 (0)	0/125 (0)	0/125 (0)	0/125 (0)	
		M + F, 123	Whole exhaust	1.8	None			0/123 (0)	4/123 (3.3)	0/123 (0)	4/123 (3.3)	
		M + F, 124	Whole exhaust	3.7	None			0/124 (0)	6/124 (4.8)	2/124 (1.6)	8/124 (6.5)[c]	
								Adenomas	Adenocarcinoma and adenosquamous carcinoma	Large cell and squamous cell carcinomas	All tumors	
Iwai et al. (1986)	Rat/F344	F, 24	Clean air	0	None	8 h/day, 7 days/week, for 24 mo	NA	1/22 (4.5)	0/22 (0)	0/22 (0)	1/22 (4.5)[f]	
		F, 24	Filtered exhaust	0	None			0/16 (0)	0/16 (0)	0/16 (0)	0/16 (0)	
		F, 24	Whole exhaust	4.9	None			3/19 (0)	3/19 (15.8)	2/19 (10.5)	8/19 (42.1)[c,g]	
									Adenoma	Carcinoma		
Takemoto et al. (1986)	Rat/F344	F, 12	Clean air	0	None	4 h/day, 4 days/week, 18-24 mo	NA		0/12 (0)	0/12 (0)		
		F, 21	Clean air	0	DIPN[h]				10/21 (47.6)	4/21 (19)		
		F, 15	Whole exhaust	2-4	None				0/15 (0)	0/15 (0)		
		F, 18	Whole exhaust	2-4	DIPN[h]				12/18 (66.7)	7/18 (38.9)		
									Adenoma	Adeno-carcinoma		
	Mouse/ IRC	M + F, 45	Clean air	0	None	4 h/day, 4 days/week, for 19-28 mo	NA		3/45 (6.7)	1/45 (2.2)		
		M + F, 69	Whole exhaust	2-4	None				6/69 (8.7)	3/69 (4.3)		
	Mouse/ C57BL	M + F, 12	Clean air	0	None	4 h/day, 4 days/week for 19-28 mo	NA		1/12 (8.3)	0/12 (0)		
		M + F, 38	Whole exhaust	2-4	None				8/38 (21.1)	3/38 (7.9)		

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]	Comments
								Primary lung tumors	
Brightwell et al. (1989)	Rat/344	M + F, 260	Clean air	0	None	16 h/day, 5 days/week, for 24 mo	NA	3/260 (1.2)	Tumor incidence for all rats dying or sacrificed
		M + F, 144	Filtered exhaust (medium exposure)	0	None			0/144 (0)	
		M + F, 143	Filtered exhaust (high exposure)	0	None			0/143 (0)	
		M + F, 143	Whole exhaust	0.7	None			1/143 (0.7)	♀ 24/25 (96%) after 24 mo ♂ 12/27 (44%) after 24 mo
		M + F, 144	Whole exhaust	2.2	None			14/144 (9.7)[c]	
		M + F, 143	Whole exhaust	6.6	None			55/143 (38.5)[c]	
								Primary lung tumors	
	Hamster/ Syrian Golden	M + F,	Clean air	0	None	16 h/day, 5 days/week, for 24 mo	NA	7/202 (3.5)	Respiratory tract tumors not related to exhaust exposure for any of the groups
		M + F, 202	Clean air	0	DEN[j]			4/104 (3.8)	
		M + F, 104	Filtered exhaust (medium dose)	0	DEN[j]			9/104 (8.7)	
		M + F, 104	Filtered exhaust (high dose)	0	DEN[j]			2/101 (2.0)	
		M + F, 101	Whole exhaust	0.7	DEN[j]			6/102 (5.9)	
		M + F, 102	Whole exhaust	2.2	DEN[j]			4/101 (3.9)	
		M + F, 101	Whole exhaust	6.6	DEN[j]			1/204 (0.5)	
		M + F, 204	Filtered exhaust (high dose)	0	None			0/203 (0)	
		M + F, 203	Whole exhaust	6.6	None				
								Adenomas	
Karagianes et al. (1981)	Rat/ Wistar	M, 40	Clean air	0	None	6 h/day, 5 days/week, for up to 20 mo	NA	0/6 (0)	
		M, 40	Whole exhaust	8.3	None			1/6 (16.6)	

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]	Comments
								Lung Tumors	
Orthoefer et al. (1981) (Pepelko and Peirano, 1983)	Mouse/ Strong A	M, 25	Clean air	0	None	20 h/day, 7 days/week, for 7 weeks		3/22 (13.6)	0.13 Tumors/ mouse
			Whole exhaust	6.4	None		26 weeks	7/19 (36.8)	0.63 Tumors/ mouse
			Whole exhaust	6.4	UV irradiated		26 weeks	6/22 (27.3)	0.27 Tumors/ mouse
								Lung Tumors	
	Mouse/ Jackson A	M + F, 40	Clean air	0	None	20 h/day, 7 days/week, for 8 weeks	8 weeks	16/36 (44.4)	0.5 Tumors/ mouse
		M + F, 40	Whole exhaust	6.4	None		8 weeks	11/34 (32.3)	0.4 Tumors/ mouse
	Mouse/ Jackson A	F, 60	Clean air	0	None	20 h/day, 7 days/week, for approx. 7 mo.		4/58 (6.9)	0.09 Tumors/ mouse
		F, 60	Clean air	0	Urethan[l]			9/52 (17.3)	0.25 Tumors/ mouse
		F, 60	Whole exhaust	6.4	None			14/56 (25.0)	0.32 Tumors/ mouse
		F, 60	Whole exhaust	6.4	Urethan[k]			22/59 (37.3)	0.39 Tumors/ mouse
		M, 429	Clean air	0	None			73/403 (18.0)	0.23 Tumors/ mouse
		M, 430	Whole exhaust	6.4	None			66/368 (17.9)	0.20 Tumors/ mouse

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]			Comments
								Adenomas	Carcinomas	All tumors	
Pepelko and Peirano (1983)	Mouse/ Sencar	M + F, 260	Clean air	0	None	Continuous	NA	(5.1)	(0.5)	(5.6)	
			Clean air	0	BHT[l]	for 15 mo		(12.2)	(1.7)	(2.8)	
			Clean air	0	Urethan[k]			(8.1)	(0.9)	(9.0)	
			Whole exhaust	12	None			(10.2)[c]	(1.0)	(11.2)[c]	
			Whole exhaust	12	BHT[l]			(5.4)	(2.7)	(8.1)	
			Whole exhaust	12	Urethan[l]			(8.7)	(2.6)	(11.2)	
									All tumors		
	Mouse/ Strain A	M + F, 90	Clean air	0	None		NA		21/87 (24)		0.29 Tumors/ mouse
			Clean air	0	Exposure (darkness)				59/237 (24.9)		0.27 Tumors/ mouse
			Whole exhaust	12	Exposure				10/80 12.5)		0.14
			Whole exhaust	12	(darkness)				22/250 (0.10)		0.10
			Clean air	0	Urethan[m]				66/75 (88)		2.80
			Whole exhaust	12	Urethan[m]				42/75 (0.95)		0.95
									Broncho-alveolar carcinoma		
Kaplan et al. (1983) White et al. (1983)	Rat/F344	M, 30	Clean air	0	None	20 h/day,	8 mo		0/30 (0)		
		M, 30	Whole exhaust	0.25	None	7 days/week,	8 mo		1/30 (3.3)		
		M, 30	Whole exhaust	0.75	None	for up to	8 mo		3/30 (10.0)		
		M, 30	Whole exhaust	1.5	None	15 mo	8 mo		1/30 (3.3)		
									Pulmonary adenoma		
	Mouse/ A/J	M, 388	Clean air	0	None	20 h/day,	NA		130/388 (33.5)		
		M, 388	Whole exhaust	0.25	None	7 days/week,			131/388 (33.8)		
		M, 399	Whole exhaust	0.75	None	for up to			109/399 (27.3)		
		M, 396	Whole exhaust	1.5	None	8 mo			99/396 (25.0)		
									Pulmonary adenomas		
Kaplan et al. (1982)	Mouse/ A/J	M, 458	Clean air	0	None	20 h/day,	6 mo		144/458 (31.4)		
		M, 18	Clean air	0	Urethan[k]	7 days/week,			18/18 (100)		
		M, 485	Whole exhaust	1.5	None	for 3 mo			165/485 (34.2)		

Table 7-1. Summary of animal carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Postexposure observation	Tumor type and incidence (%)[a]					Comments
								Adenomas	Carcinomas	All tumors			
Ishinishi et al. (1988a)	Rat/F344	NS, 5	Whole exhaust	0.1	None	16 h/day,	6 mo	0/5 (0)	0/5 (0)	0/5 (0)			
		NS, 8	Whole exhaust	0.1	None	6 days/week,	12 mo	0/8 (0)	0/8 (0)	0/8 (0)			
		NS, 11	Whole exhaust	0.1	None	for 12 mo	18 mo	0/11 (0)	0/11 (0)	0/11 (0)			
Light duty		NS, 5	Whole exhaust	1.1	None		6 mo	0/5 (0)	0/5 (0)	0/5 (0)			
		NS, 9	Whole exhaust	1.1	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)			
		NS, 11	Whole exhaust	1.1	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)			
Heavy duty		NS, 5	Whole exhaust	0.5	None	16 h/day,	6 mo	0/5 (0)	0/5 (0)	0/5 (0)			
		NS, 9	Whole exhaust	0.5	None	6 days/week,	12 mo	0/9 (0)	0/9 (0)	0/9 (0)			
		NS 11	Whole exhaust	0.5	None	for 12 mo	18 mo	0/11 (0)	0/11 (0)	0/11 (0)			
		NS, 5	Whole exhaust	1.8	None		6 mo	0/5 (0)	0/5 (0)	0/11 (0)			
		NS, 6	Whole exhaust	1.8	None		12 mo	0/6 (0)	0/6 (0)	0/6 (0)			
		NS, 13	Whole exhaust	1.8	None		18 mo	0/13 (0)	1/13 (0)	1/13 (0)			
Lewis et al. (1989)	Rat/F344	M + F, 288[n]	Clean air	0	None	7 h/day,	NA	No tumors		0/192 (0)			
			Whole exhaust	2.0	None	5 days/week, 24 mo[o]				0/192 (0)			
								Multiple adenomas	Multiple carcinomas	Adenomas/ carcinoma	Alveolar/ bronchiolar adenoma	Alveolar/ bronchiolar carcinoma	
Mauderly et al. (1996)	Mouse/ CD-1	M + F	Clean air	0	None	7 h/day, 5	None	1/157 (0.6)	2/157 (1.3)	1/157 (0.6)	10/157 (6.4)	7/157 (4.5)	
		M + F	Whole exhaust	0.35	None	days/week,		2/171 (1.2)	1/171 (0.6)	1/171 (0.6)	16/171 (9.4)	5/171 (2.9)	
		M + F	Whole exhaust	3.5	None	for up to 24		0/155 (0)	1/155 (0.6)	0/155 (0)	8/155 (5.2)	6/155 (3.9)	
		M + F	Whole exhaust	7.0	None	mo		0/186 (0)	0/186 (0)	0/186 (0)	10/186 (5.4)	4/186 (2.2)	

[a]Table values indicate number with tumors/number examined (% animals with tumors).
[b]Number of animals examined for tumors.
[c]Significantly different from clean air controls.
[d]Diphenylnitrosamine; 6.25 mg/kg/week s.c. during first 25 weeks of exposure.
[e]Diphenylnitrasamine; 12.5 mg/kg/week s.c. during first 25 weeks of exposure.
[f]Splenic lymphomas also detected in controls (8.3%), filtered exhaust group (37.5%) and whole exhaust group (25%).
[g]5.3% incidence of large cell carcinomas.
[h]1 g/kg, i.p. 1/week for 3 weeks starting 1 mo into exposure.
[i]Includes adenomas, squamous cell carcinomas, adenocarcinomas, adenosquamous cell carcinoma, and mesotheliomas.
[j]4.5 mg/DEN/kg, s.c., 3 days prior to start of inhalation exposure.
[k]Single i.p. dose 1 mg/kg at start of exposure.
[l]Butylated hydroxytoluene 300 mg/kg, i.p. for week 1, 83 mg/kg for week 2, and 150 mg/kg for weeks 3 to 52.
[m]12 mg/m^3 from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 mg/m^3.
[n]120-121 males and 71-72 females examined histologically.
[o]Not all animals were exposed for full term, at least 10 males were killed at 3, 6, and 12 mo of exposure.
NS = Not specified.

NA = Not applicable.

respectively). Bronchoalveolar adenomas, adenocarcinomas, and squamous cysts (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 of 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 ($p<0.001$) exposure-response relationship was obtained for tumor incidence relative to exposure concentration and lung burden of DPM. These data are summarized in Table 7-1. A logistic regression model estimating tumor prevalence as a function of time, dose (lung burden of DPM), and sex indicated a sharp increase in tumor prevalence for the high dose level at about 800 days after the commencement of exposure. A less pronounced, but definite, increase in prevalence with time was predicted for the medium-dose level. Significant effects were not detected at the low concentration. DPM (mg per lung) of rats exposed to 0.35, 3.5, or 7.1 mg of DPM/m^3 for 24 mo were 0.6, 11.5, and 20.8, respectively, and affirmed the greater than predicted accumulation that was the result of decreased particle clearance following high-exposure conditions.

In summary, this study demonstrated the pulmonary carcinogenicity of high concentrations of whole, diluted diesel exhaust in rats following chronic inhalation exposure. In addition, increasing lung particle burden resulting from this high-level exposure and decreased clearance was demonstrated. A logistic regression model presented by Mauderly et al. (1987) indicated that both lung DPM burden and exposure concentration may be useful for expressing exposure-effect relationships.

A series of studies was conducted at the Fraunhofer Institute of Toxicology and Aerosol Research in which female Wistar rats were exposed for 19 h/day, 5 days/week to both filtered and unfiltered (total) diesel exhaust at an average particulate matter concentration of 4.24 mg/m^3. Animals were exposed for a maximum of 2.5 years. 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%. 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 aerodynamic diameter of DPM was 0.35 ± 0.10 μm (mean ± SD). The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A.

The effects of exposure to either filtered or unfiltered exhaust were described by Heinrich et al. (1986a) and Stöber (1986). Exposure to unfiltered exhaust resulted in 8 bronchoalveolar

adenomas and 9 squamous cell tumors in 15 of 95 rats examined for a 15.8% tumor incidence. Although statistical analysis was not provided, the increase appears to be highly significant. In addition to the bronchioalveolar adenomas and squamous cell tumors, there was a high incidence of bronchioalveolar hyperplasia (99%) and metaplasia of the bronchioalveolar epithelium (65%). No tumors were reported among female Wistar rats exposed to filtered exhaust (n = 92) or clean air (n = 96).

Mohr et al. (1986) provided a more detailed description of the lung lesions and tumors identified by Heinrich et al. (1986a, 1986b) and Stöber (1986). Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to the unfiltered diesel exhaust. Squamous metaplasia was observed in 65.3% of the rats breathing unfiltered diesel exhaust but not in the control rats. Of nine squamous cell tumors, one was characterized as a Grade I carcinoma (borderline atypia, few to moderate mitoses, and slight evidence of stromal invasion), and the remaining eight were classified as benign keratinizing cystic tumors.

The effect of chronic (19 h/day, 5 days/week, 2 to 2.5 years) diesel exhaust exposure on the tumor-inducing effect of diphenylnitrosamine (DPN) was examined using female Wistar rats (Heinrich et al., 1986a; Stöber, 1986; Heinrich, 1989a). Groups of rats (45 to 48 per group) were exposed to clean air or whole diesel exhaust (particle concentration of 4.24 mg/m^3, as described previously) and administered by s.c. injection 250 or 500 mg DPN/kg/week during the first 25 weeks of exposure. The total DPN dose administered equaled 6.25 or 12.5 g/kg of body weight. The concentrations of B[a]P, benzo[e]pyrene (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 7-1). Conversely, the high level of nasal tumors induced by DPN was significantly decreased in the rats exposed to the diesel engine emissions.

Heinrich et al. (1986b) and Mohr et al. (1986) compared the effects of exposure to particles having only a minimal carbon core but a much greater concentration of PAHs than DPM does. The desired exposure conditions were achieved by mixing coal oven flue gas with pyrolyzed pitch. The concentration of B[a]P and other PAHs per milligram of DPM was about three orders of magnitude greater than that of diesel exhaust. Female rats were exposed to the flue gas-pyrolyzed pitch for 16 h/day, 5 days/week at particle concentrations of 3 to 7 mg/m^3 for 22 mo, then held in clean air for up to an additional 12 mo. Among 116 animals exposed, 22 tumors were reported in 21 animals, for an incidence of 18.1%. One was a bronchioloalveolar adenoma, one was a bronchioloalveolar carcinoma, and 20 were squamous cell tumors. Among

the latter, 16 were classified as benign keratinizing cystic tumors, and 4 were classified as carcinomas. No tumors were reported in 115 controls. The tumor incidence in this study was comparable to that reported previously for the diesel exhaust-exposed animals.

The importance of DPM and of insoluble respirable particles in tumorigenic responses of rats was investigated and reported by Heinrich et al. (1995). In this chronic inhalation exposure study, female Wistar rats were exposed to whole diesel exhaust (0.8, 2.5, or 7.0 mg/m^3), 18 h/day, 5 days/week for up to 24 mo. Groups of rats were also exposed to ultrafine TiO_2 particles (10 mg/m^3) and carbon black particles (11.6 mg/m^3) using the same exposure regimen, except that after 4 mo the exposure concentrations of TiO_2 and carbon black were increased to obtain lung particle burdens similar to those observed in rats exposed to whole diesel exhaust. Controls were exposed to clean air. Following exposure to the test atmospheres, the rats were maintained in clean air atmospheres for an additional 6 mo.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole diesel exhaust, coal oven flue gas, or carbon black (15.8%, 18.1%, and 8% to 17%, respectively) was very similar. This occurred despite the fact that the PAH content of the PAH-enriched pyrolyzed pitch was more than three orders of magnitude greater than that of diesel exhaust; carbon black, on the other hand, had only traces of PAHs. Based on these findings, the organic fraction is not the sole cause of tumor induction by diesel exhaust. This issue is discussed further in Chapter 10.

In the Heinrich et al. (1995) report, the cumulative exposures for the rats in the various treatments groups were 61.7, 21.8, and 7.4 g/m^3 × h for the high, medium, and low whole-exhaust exposures and 102.2 and 88.1 g/m^3 × h for the carbon black and TiO_2 groups, respectively. For tumor incidence comparison (number of rats with tumors) among the diesel exhaust exposure groups, significant increases were observed in the high (22/100; $p<0.001$) and mid (11/200; $p<0.01$) exposure groups relative to clean air controls (Table 7-1). Only one tumor (1/217), an adenocarcinoma, was observed in clean air controls. Relative to clean air controls, significantly increased incidences were observed in the high exposure rats for benign squamous cell tumors (14/100; $p<0.001$), adenomas (4/100; $p<0.01$), and adenocarcinomas (5/100; $p<0.05$). Only the incidence of benign squamous cell tumors (7/200; $p<0.01$) was significantly increased in the mid-exposure group relative to the clean air controls. In comparing the number of rats with tumors (including benign squamous cell tumors), incidences in the TiO_2 exposure group (32/100) were similar to that of the high diesel exhaust exposure (22/100), but the incidence in the carbon black group (39/100) was significantly greater ($p<0.01$) than that of the high diesel exhaust. For the carbon black group, the incidences of adenomas (13/100) and

adenocarcinomas (13/100) were significantly greater ($p<0.05$) than in the high diesel exhaust group. The incidence of adenocarcinomas (13/100) was also significantly greater ($p<0.05$) in the TiO_2 group than in diesel exhaust-exposed rats.

Particle lung burden and alveolar clearance also were determined in the Heinrich et al. (1995) study. Even with adjustments to the exposure protocol to increase lung particle burden, at 12 mo of exposure and beyond the particle lung burden in high diesel exhaust group (63,878 μg/animal) was greater than that of the TiO_2 (43,854 μg/animal) and carbon black (39,287 μg/animal) groups. Relative to clean air controls, alveolar clearance was significantly compromised by exposure to mid and high diesel exhaust, TiO_2, and carbon black after 3 mo of exposure. Exposure to the high concentration of diesel exhaust resulted in a greater reduction of alveolar clearance than exposure to either TiO_2 or carbon black did. For the high diesel exhaust, TiO_2, and carbon black exposure groups, the 3-mo recovery time in clean air failed to reverse the compromised alveolar clearance.

A study conducted at the Inhalation Toxicology Research Institute also compared the lung tumor response of rats chronically exposed to diesel exhaust and carbon black (Nikula et al. 1995). In this study, male (114-115 per exposure group) and female (114-116 per exposure group) F344 rats were exposed to either carbon black or diesel exhaust for 16 h/day, 5 days/week to particle concentrations of 2.5 or 6.5 mg/m^3 for up to 24 mo. Controls (118 males, 114 females) were exposed to clean air. The progressive pulmonary accumulation of DPM tended to be more rapid than that for carbon black and accumulations of both tended to accelerate after 12 mo, a finding similar to that reported by Heinrich et al. (1994). At 23 mo, mean lung burdens of females exposed to low carbon black or high carbon black were 17.3 and 36.9 mg, respectively; and for males, the lung burdens were 24.7 and 40.1 mg, respectively. For low and high diesel exhaust exposure, the lung burdens were 36.7 and 80.7 mg, respectively, for females and 45.1 and 90.1 mg, respectively, for males. Both diesel exhaust and carbon black were pulmonary carcinogens under the exposure conditions of the study. The percentages of susceptible rats (males and females combined) with malignant neoplasms were 0.9 (control), 3.8 (low carbon black), 11.8 (high carbon black), 3.3 (low diesel exhaust), and 12.3 (high diesel exhaust). The percentages of rats (males and females combined) with malignant or benign neoplasms were 1.4 (control), 4.7 (low carbon black), 15.2 (high carbon black), 6.2 (low diesel exhaust), and 17.9 (high diesel exhaust). All primary neoplasms were associated with the parenchyma rather than the conducting airways of the lungs. The first lung neoplasm was observed at 15 mo. The specific tumor types and incidences are shown in Table 7-1. Analysis of the histopathologic data suggested a progressive process from alveolar epithelial hyperplasia to adenomas and adenocarcinomas. The neoplastic responses to carbon black and diesel exhaust were similar,

indicating that the organic fraction adsorbed to the DPM was not contributing significantly to the carcinogenic response. Although these data provide indirect evidence that the DPM-associated organic fraction of diesel exhaust did not play a significant role in the observed carcinogenic response, the data do not prove that the organic fraction has no role whatsoever. However, if DPM-associated organics are involved, the great difference in organic fraction content between carbon black and diesel exhaust (i.e., three orders of magnitude) suggests that its role was minor in the tumorigenic response of the rats in this study.

A long-term inhalation study (Ishinishi et al., 1988a; Takaki et al., 1989) examined the effects of emissions from light-duty (LD) and heavy-duty (HD) diesel engines on male and female Fischer 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 1,200 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/day, 6 days/week) used DPM concentrations of 0, 0.5, 1, 1.8, or 3.7 mg/m^3 from HD engines and 0, 0.1, 0.4, 1.1, or 2.3 mg/m^3 from LD engines. The B[a]P 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 is presented in Appendix A. The animals inhaled the exhaust emissions from 1700 to 0900 h. Sixty-four male rats and 59 to 61 female rats from each exposure group were evaluated for carcinogenicity.

For the experiments using the LD series engines, the highest incidence of hyperplastic lesions plus tumors (72.6%) was seen in the highest exposure (2.3 mg/m^3) group. However, this high value was the result of the 70% incidence of hyperplastic lesions; the incidence of adenomas was only 0.8% and that of carcinomas 1.6%. Hyperplastic lesion incidence was considerably lower for the lower exposure groups (9.7%, 4.8%, 3.3%, and 3.3% for the 1.1, 0.4, and 0.1 mg/m^3 and control groups, respectively). The incidence of adenomas and carcinomas, combining males and females, was not significantly different among exposure groups (2.4%, 4.0%, 0.8%, 2.4%, and 3.3% for the 2.3, 1.1, 0.4, and 0.1 mg/m^3 groups and the controls, respectively).

For the experiments using the HD series engines, the total incidence of hyperplastic lesions, adenomas, and carcinomas was highest (26.6%) in the 3.7 mg/m^3 exposure group. The incidence of adenomas plus carcinomas for males and females combined equaled 6.5%, 3.3%, 0%, 0.8%, and 0.8% at 3.7, 1.8, 1, and 0.4 mg/m^3 and for controls, respectively. A statistically significant difference was reported between the 3.7 mg/m^3 and the control groups for the HD

series engines. A progressive dose-response relationship was not demonstrated. Tumor incidence data for this experiment are presented in Table 7-1.

The Ishinishi et al. (1988a) study also included recovery tests in which rats exposed to whole diesel exhaust (DPM concentration of 0.1 or 1.1 mg/m^3 for the LD engine and 0.5 or 1.8 mg/m^3 for the HD engine) for 12 mo were examined for lung tumors following 6-, 12-, or 18-mo recovery periods in clean air. The incidences of neoplastic lesions were low, and pulmonary DPM burden was lower than for animals continuously exposed to whole diesel exhaust and not provided a recovery period. The only carcinoma observed was in a rat examined 12 mo following exposure to exhaust (1.8 mg/m^3) from the HD engine.

Iwai et al. (1986) also examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 2.4 L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20°C to 25°C and 50% relative humidity. The engines were operated at 1,000 rpm with an 80% engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest NO_2 and SO_2 content. For those chambers using filtered exhaust, proximally installed high-efficiency particulate air (HEPA) filters were used. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air for 8 h/day, 7 days/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-year control (filtered room air) rats, although one adenoma was noted in a 30-mo control rat, providing a spontaneous tumor incidence of 4.5%. No lung tumors were observed in rats exposed to filtered diesel exhaust. Four of 14 rats exposed to unfiltered diesel exhaust for 2 years developed lung tumors, two of these were malignant. Five rats of this 2-year exposure group were subsequently placed in clean room air for 3 to 6 mo and four eventually (time not specified) exhibited lung tumors (three malignancies). Thus, the lung tumor incidence for total tumors was 42.1% (8/19) and 26.3% (5/19) for malignant tumors in rats exposed to whole diesel exhaust. The tumor types identified were adenoma (3/19), adenocarcinoma (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 7-1. Malignant splenic lymphomas were detected in 37.5% of the rats in the filtered exhaust group and in 25.0% of the rats in the unfiltered exhaust group, these values were significantly ($p \leq 0.05$) greater than the 8.2% incidence noted in the control rats. The study demonstrates production of lung cancer in rats following 2-year exposure to unfiltered diesel exhaust. In addition, splenic malignant lymphomas occurred during exposure to both filtered and unfiltered

diesel exhaust. This is the only report to date of tumor induction at an extrarespiratory site by inhaled diesel exhaust in animals.

A chronic (up to 24 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 of female F344/Jcl rats. One mo after initiation of inhalation exposures, DIPN was administered i.p. at 1 mg/kg weekly for 3 weeks to clean air- and diesel-exposed groups of rats. Uninjected groups were also exposed to clean air and diesel exhaust. The treatment protocol consisted of exposure to diesel exhaust for 4 h/day, 4 days/week. The diesel exhaust was generated by a 269-cc engine operated at an idle state (1,600 rpm). Concentrations of the gas-phase components of the exhaust are presented in Appendix A. The particle concentration of the diesel exhaust in the exposure chamber was 2 to 4 mg/m^3. B[a]P and 1-nitropyrene concentrations were 0.85 and 93 μg/g of particles, respectively.

In the Takemoto et al. (1986) study, no lung tumors were reported in either uninjected controls or diesel-exposed animals. Among injected animals autopsied at 12 to 17 mo, 2 adenomas were reported in 8 rats exposed to clean air compared with 12 adenomas and 3 adenocarcinomas in 18 diesel-exposed rats. Among injected rats autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were seen in 21 animals exposed to clean air compared with 12 adenomas and 7 adenocarcinomas in 18 diesel-exposed rats. According to the authors, the incidence of malignant tumors was not significantly increased in either of the diesel exhaust-exposed groups when compared with the appropriate control group. Tumor incidence data for the various treatment protocols are presented in Table 7-1. It was also noted that the diesel engine employed in this study was originally used as an electrical generator and that its operating characteristics (not specified) were different from those for a diesel-powered automobile. However, the investigators deemed it suitable for assessing the of effects diesel emissions.

Brightwell et al. (1986, 1989) studied the effects of filtered and unfiltered diesel exhaust on male and female F344 rats. The diesel exhaust was generated by a 1.5-L Volkswagen engine that was computer-operated according to the U.S. 72 FTP driving cycle. The engine emissions were diluted by conditioned air delivered at 800 m^3/h to 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) and low- (0.7 mg/m^3) exposure atmospheres. Filtered diesel exhaust was generated by a similar engine. The CO and NO_x concentrations (mean ± SD) were 32 ± 11 ppm and 8 ± 1 ppm for the unfiltered diesel exhaust (high-exposure concentration chamber) and 32 ± 11 and 8 ± 1 for the filtered diesel exhaust. The inhalation exposures were conducted overnight to provide five 16-h periods per week for 2 years; surviving animals were maintained for an additional 6 mo.

For males and females combined, a 9.7% (14/144) and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to 2.2 and 6.6 mg of DPM/m^3, respectively (Table 7-1). The tumor incidence in the 0.7 mg/m^3 exposure group was 0.7% (1/144) and that of controls was 1.2% (3/260). Diesel exhaust-induced tumor incidence in rats was dose-related and higher in females than in males (Table 7-1). These data included animals sacrificed at the interim periods (6, 12, 18, and 24 mo); therefore, the tumor incidence does not accurately reflect the effects of long-term exposure to the diesel exhaust atmospheres. When tumor incidence is expressed relative to the specific intervals, a lung tumor incidence of 96% (24/25), 76% (19/25) of which were malignant, was reported for female rats in the high dose group exposed for 24 mo and held in clean air for the remainder of their lives. For male rats in the same group, the tumor incidence equaled 44% (12/27), of which 37% (10/27) were malignant. It was also noted that many of the animals exhibiting tumors had more than one tumor, often representing multiple histological types. The types of tumors identified in the rats exposed to diesel exhaust included adenomas, squamous cell carcinomas, adenocarcinomas, mixed adenoma/adenocarcinomas, and mesotheliomas. Similar to other studies, the tumor incidence in rats occurred during exposure to whole exhaust rather than filtered exhaust. It must be noted, however, that the exposure during darkness (when increased activity would result in greater respiratory exchange and greater inhaled dose) could account, in part, for the high response reported for the rats.

Karagianes et al. (1981) exposed male Wistar rats (40 per group) to diesel engine exhaust diluted to a DPM concentration of 8.3 (± 2.0) mg/m^3, room air, diesel engine exhaust (8.3 mg/m^3) plus low-concentration coal dust (5.8 mg/m^3), low-concentration coal dust only (6.6 mg/m^3), or high-concentration coal dust (14.9 mg/m^3) 6 h/day, 5 days/week for up to 20 mo. The exhaust-generating system and exposure atmosphere characteristics are presented in Appendix A. The type of engine used (3-cylinder, 43 bhp diesel) is normally used in mining situations and was connected to an electric generator and operated at varying loads and speeds to simulate operating conditions in an occupational situation. To control the CO concentration at 50 ppm, the exhaust was diluted 35:1 with clean air.

One bronchiolar adenoma was detected in the group exposed to diesel exhaust alone and one in the rats receiving combined exposures. No lung tumors were reported in controls or following exposure to either high or low concentrations of coal dust. The equivocal response may have been caused by the relatively short exposure durations (20 mo). In the Mauderly et al. (1987) study, by comparison, most of the tumors were detected in rats exposed for more than 24 mo.

Lewis et al. (1989) also examined the effects of inhalation exposure of diesel exhaust and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole diesel exhaust (2 mg soot/m^3), coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), or diesel exhaust plus coal dust (1 mg/m^3 of each respirable concentration; 3.2 mg/m^3 total concentration) for 7 h/day, 5 days/week for up to 24 mo. Groups of 10 or more males were sacrificed at intermediate intervals (3, 6, and 12 mo). The diesel exhaust was produced by a 7.0-L, 4-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel (<0.5% sulfur by mass). The exhaust was passed through a Wagner water scrubber, which lowered the exhaust temperature and quenched engine backfire. An analysis of the exposure atmospheres is presented in Appendix A.

Histological examination was performed on 120 to 121 male and 71 to 72 female rats terminated after 24 mo of exposure. The exhaust exposure did not significantly affect the tumor incidence beyond what would be expected for aging F344 rats. There was no postexposure period, which may explain, in part, the lack of significant tumor induction. The particulate matter concentration was also less than the effective dose in several other studies.

General Motors Research Laboratories sponsored chronic inhalation studies using male Fischer 344 rats exposed to DPM concentrations of 0.25, 0.75, or 1.5 mg/m^3 (Kaplan et al., 1983; White et al., 1983). The exposure protocol for this study conducted at the Southwest Research Institute was 20 h/day, 7 days/week for 9 to 15 mo. Some animals were sacrificed following completion of exposure, while others were returned to clean air atmospheres for an additional 8 mo. Control animals received clean air. Exhaust was generated by 5.7-L Oldsmobile engines (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. Details of the exhaust-generating system and exposure atmosphere are presented in Appendix A.

Five instances of bronchoalveolar carcinoma were observed in 90 rats exposed to diesel exhaust for 15 mo and held an additional 8 mo in clean air. These included one tumor in the 0.25 mg/m^3 group, three in the 0.75 mg/m^3 group, and one in the 1.5 mg/m^3 group. Rats kept in clean air chambers for 23 mo did not exhibit any carcinomas. No tumors were observed in any of the 180 rats exposed to diesel exhaust for 9 or 15 mo without a recovery period or in the respective controls for these groups. Although the increases in tumor incidences in the groups exposed for 15 mo and held an additional 8 mo in clean air were not statistically significant, they suggest an effect because the background incidence for this specific lesion in this strain of rat is low.

7.2.2. Mouse Studies

Heinrich et al. (1986a) and Stöber (1986), as part of a larger study, also evaluated the effects of diesel exhaust in mice. Details of the exposure conditions reported by Heinrich et al. (1986b) are given in Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to filtered (n = 93) and unfiltered (n = 76) diesel exhaust (4.2 mg/m^3), female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 31% (filtered) and 32% (unfiltered), respectively. Tumor incidences reported for control mice (n = 84) equaled 11% for adenomas and adenocarcinomas combined. The effects are more dramatic when the incidences of only malignant tumors (adenocarcinomas) are considered, 2.4% for controls, 19% for filtered exhaust, and 17% for unfiltered exhaust. This is the only reported study in which filtered exhaust resulted in a definitive tumorigenic response in the lungs of mice. These data are summarized in Table 7-1.

As part of the same study, groups of 64 female NMRI mice of 8 to 10 weeks of age were dosed weekly with either 50 or 100 μg B[a]P intratracheally for 20 or 10 weeks, respectively, for a total dose of 1 mg. Another group received 50 μg dibenz[*a,h*]anthracene (DBA) intratracheally for 10 weeks. Additional groups of 96 newborn mice received one s.c. injection of 5 or 10 μg of DBA between 24 and 48 h after birth. The animals were concomitantly exposed to either diesel exhaust or clean air. The mice receiving intratracheal instillations were observed throughout their lifespan but the newborn mice were sacrificed after 6 mo. Although the chemical treatments resulted in large increases in lung tumor incidence, exposure to diesel exhaust did not enhance this effect and in some cases even resulted in inhibition. For example, lung tumor rates in clean air mice treated with 20 instillations of B[a]P equaled 71% compared with 41% for mice similarly instilled but exposed to diesel exhaust. The decrease resulted from a smaller number of adenocarcinomas, whereas the adenoma incidence remained unchanged. The high dose of DBA injected into newborn mice also resulted in a greater tumor incidence in mice exposed to clean air (81%) than in the diesel exposed group (63%). Effects of the other treatments were apparently not inhibited by diesel exhaust exposure, although complete incidence data were not reported. The authors did not speculate on the reasons for this unexpected effect.

In addition to the studies using rats, investigators at the Fraunhofer Institute also examined the effects in mice following long-term exposure to whole diesel exhaust, TiO_2, and carbon black particles (Heinrich et al., 1995). In these studies, NMRI mice were exposed (18 h/day, 5 days/week) to whole diesel exhaust (4.5 mg/m^3 for 13.5 mo), TiO_2 (7.0 mg/m^3 for 4 mo followed by 15.0 mg/m^3 for 4 mo and 10 mg/m^3 for 5.5 mo), or carbon black (7.0 mg/m^3 for 4 mo followed by 12 mg/m^3 for 9.5 mo). Following the 13.5-mo exposures, animals were kept in clean air for a total experiment time of 23 mo. Controls were exposed to clean air. The lung

burdens of the NMRI mice after 12 mo of exposure were 7.0, 7.4, and 5.2 mg/lung for DPM, carbon black, and TiO_2, respectively. Adenomas and adenocarcinomas were the only tumor types observed in the mice. Percentages for adenomas/adenocarcinomas were 21.8%/15.4% for whole diesel exhaust, 11.3%/10% for carbon black, 11.3%/2.5% for TiO_2, and 25%/15.4% for clean air controls. The lung tumor rates for adenomas/adenocarcinomas were 32.1% (diesel exhaust), 20% (carbon black), 13.8% (TiO_2), and 30% (clean air controls).

A comparison between particle-free exhaust and whole exhaust was also conducted using NMRI and C57BL mice (Heinrich et al., 1995). In the experiments with NMRI mice, mice were exposed to whole diesel exhaust (4.5 mg/m^3) and another group was exposed to particle-free diesel exhaust for 23 mo. The lung tumor rates for the groups of mice were 23% (whole exhaust), 46.7% (particle-free exhaust), and 30% (clean air controls). The difference between the controls and those exposed to the particle-free exhaust was not significant (p=0.053). The percentages of adenomas/adenocarcinomas were 18.3%/5% (whole exhaust), 31.7%/15% (particle-free exhaust), and 25%/8.8% (clean air controls). In the experiments using C57BL mice, the exposure groups were similar except that the exposure duration was 24 mo. The tumor rates (including an additional 6-mo exposure in clean air) were not significantly different among the whole exhaust (8.5%), particle-free exhaust (3.5%), and clean air controls (5.1%).

The lack of a carcinogenic response in mice was reported by Mauderly et al. (1996). In this study, groups of 540 to 600 CD-1 male and female mice were exposed to whole diesel exhaust (7.0, 3.5, or 0.35 mg DPM/m^3) for 7 h/day, 5 days/week for up to 24 mo. Controls were exposed to filtered air. DPM accumulation in the lungs of exposed mice was assessed at 6, 12, and 18 mo of exposure and was shown to be progressive; DPM burdens were 0.2 ± 0.02, 3.7 ± 0.16, and 5.6 ± 0.39 mg for the low-, medium-, and high-exposure groups, respectively. The lung burdens in both the medium- and high-exposure groups exceeded that predicted by exposure concentration ratio to the low-exposure group. Contrary to what was observed in rats (Heinrich et al., 1986a; Stöber, 1986; Nikula et al., 1995; Mauderly et al., 1987), an exposure-related increase in primary lung neoplasms was not observed in the CD-1 mice, supporting the contention of a species difference in the pulmonary carcinogenic response to poorly soluble particles. The percentage incidence of mice (males and females combined) with one or more malignant or benign neoplasms was 13.4, 14.6, 9.7, and 7.5 for controls and low-, medium-, and high-exposure groups, respectively.

Takemoto et al. (1986) reported the effects of inhaled diesel exhaust (2 to 4 mg/m^3, 4 h/day, 4 days/week, for up to 28 mo) in ICR and C57BL mice exposed from birth. Details of the exposure conditions are presented in Appendix A. Among male and female ICR mice autopsied at 13 to 18 mo, 4 adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-exposed

mice compared with 3 adenomas among 38 controls. Among animals autopsied at 19 to 28 mo, 6 adenomas and 3 adenocarcinomas were seen in 22 exposed animals compared with 3 adenomas and one adenocarcinoma in 22 controls. Among combined male and female C57BL mice autopsied at 13 to 18 mo, 4 adenomas and 2 adenocarcinomas were detected in 79 animals autopsied compared with none in 19 unexposed animals. Among males and females autopsied at 19 to 28 mo, 8 adenomas and 3 adenocarcinomas were detected in 71 exposed animals compared with 1 adenoma among 32 controls. No significant increases in either adenoma or adenocarcinoma incidences were reported for either strain of exposed mice. Although not tested by the authors, the combined incidence of adenomas and adenocarcinomas (11/71) in male and female C57BL mice exposed to diesel exhaust for 19 to 28 mo versus that found in controls (1/32), however, appears to be a significant increase. Although the results are not definitive, there is the strong suggestion of an effect, especially since the C57BL strain has a low background lung tumor incidence. See Table 7-1 for details of tumor incidence.

Pepelko and Peirano (1983) summarized a series of studies on the health effects of diesel emissions in mice. 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. Details of the exhaust-generating system and the exposure atmosphere are presented in Appendix A. Sixty-day pilot studies were conducted at a 1:14 dilution, providing DPM concentrations of 6 mg/m^3. The engines were operated using the Modified California Cycle. These 20-h/day, 7-days/week pilot studies using rats, cats, guinea pigs, and mice produced decreases in weight gain and food consumption. Therefore, at the beginning of the long-term studies, exposure time was reduced to 8 h/day, 7 days/week at an exhaust DPM concentration of 6 mg/m^3. During the final 12 mo of exposure, however, the DPM concentration was increased to 12 mg/m^3. For the chronic studies, the engines were operated using the Federal Short Cycle.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust alone or treated with either tumor initiators or promoters. Male and female parent generation mice were exposed to diesel exhaust at a DPM concentration of 6 mg/m^3 prior to (from weaning to sexual maturity) and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) received i.p. injections of either 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 at 6 weeks of age, or no injections. The exhaust exposure was increased to a DPM 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 (16.3%) was significantly increased in the non-injected female mice exposed to diesel exhaust compared with 6.3% in clean air controls. The incidence in males and females combined was 10.2% in 205 animals examined compared with 5.1% in 205 clean air controls. This difference was also significant. The incidence of carcinomas was not affected by exhaust exposure in either sex. Exhaust exposure reduced the adenoma incidence in female mice receiving BHT (3.9% vs. 16.7%). The response to BHT in males or urethan in both sexes was unaffected by diesel exposure. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The limited response may well have been influenced by the relatively early sacrifice times of the mice. On the other hand, an increase in the sensitivity of the study, allowing detection of tumors at 15 mo, may have been the result of exposure from conception. It is interesting to note that in this study diesel exposure appeared to inhibit effects of tumor promotion, whereas Stöber (1986) reported diesel exposure inhibition of complete carcinogens. These data are summarized in Table 7-1.

A series of inhalation studies using strain A mice was conducted by Orthoefer et al. (1981). In assays with the strain A, mice are usually given a series of intraperitoneal injections with the test agent; they are then sacrificed at about 9 mo of age and examined for lung tumors. In the present series, inhalation exposure was substituted. In the current series, groups of 25 male Strong A mice were exposed to irradiated (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m^3) for 20 h/day, 7 days/week for 7 weeks. Additional groups of 40 Jackson A (20 of each sex) were exposed similarly to either clean air or diesel exhaust, then held in clean air until sacrificed at 9 mo of age. No tumorigenic effects were detected at 9 mo of age. Further studies were conducted in which male A/Strong mice were exposed 8 h/day, 7 days/week until sacrifice (approximately 300 at 9 mo of age and approximately 100 at 12 mo of age). With the exception of those treated with urethan, the number of tumors per mouse did not exceed historical control levels in any of the studies. Exposure to diesel exhaust, however, significantly inhibited the tumorigenic effects of the 5-mg urethan treatment. Results are listed in Table 7-1.

Kaplan et al. (1982) also reported the effects of diesel exposure in strain A mice. Groups of male strain A/J mice were exposed for 20 h/day, 7 days/week for 90 days and held until 9 mo of age. Experimental conditions are described in Appendix A. Briefly, the animals were exposed to diesel exhaust at DPM concentrations of 0, 0.25, 0.75, or 1.5 mg/m^3. Controls were exposed to clean air. Among 458 controls and 485 exposed animals, tumors were detected in 31.4% of those breathing clean air versus 34.2% of those exposed to diesel exhaust. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to diesel exhaust for 8 mo (Kaplan et al., 1983; White et al., 1983). After exposure to the highest exhaust concentration (1.5 mg/m^3), the percentage of mice with pulmonary adenomas and the mean number of tumors per mouse were significantly less (p<0.05) than those for controls (25.0% vs. 33.5% and 0.30 ± 0.02 [S.E.] vs. 0.42 ± 0.03 [S.E.]) (Table 7-1).

7.2.3. Hamster Studies

Heinrich et al. (1982) examined the effects of diesel exhaust exposure on the tumor frequency in female Syrian golden hamsters pretreated with the tumor initiators dibenzo[a,h]anthracene (DBA) or diethylnitrosamine (DEN). At the time of this work, it was presumed that traditional inhalation exposure experiments would not result in definitive tumor formation; thus a tumor initiation animal model was used. Groups of 48 to 72 animals were exposed to clean air, whole diesel exhaust at a mean DPM concentration of 3.9 mg/m^3, or filtered diesel exhaust with either no further treatment or administered DBA (itr. instillations of 0.1 mg/week for 20 weeks), DEN (1.5 or 4.5 mg/kg s.c.), or pyrene (itr. instillations of 0.1 mg/week for 20 weeks), the last serving as a noncarcinogenic PAH control. Inhalation exposures were conducted 7 to 8 h/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated at 2,400 rpm.

Only two hamsters exhibited lung tumors, both having died during the exposure period. One tumor occurred in a hamster receiving DBA and exposed to filtered diesel exhaust for 75 weeks; the other tumor occurred in a hamster receiving DEN and exposed to whole diesel exhaust for 67 weeks. Compared with corresponding treatment groups, there was a higher incidence of adenomatous proliferative changes in the lungs of hamsters exposed to whole diesel exhaust. Hamsters exposed to filtered diesel exhaust also showed a greater incidence of adenomatous proliferative changes than those of the respective clean air exposure groups did. The incidence of proliferative changes in the lungs of hamsters receiving DEN or DBA was greater than for those groups not treated with the initiators. Although not definitive, this study provided information suggesting the possible involvement of whole diesel exhaust and filtered diesel exhaust in producing histologic alterations in the lungs of hamsters, though no increases in tumors were observed.

In a more recent study, Syrian hamsters were exposed 19 h/day, 5 days/week for a lifetime to diesel exhaust diluted to a DPM concentration of 4.24 mg/m^3 (Heinrich et al., 1986a; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air, whole exhaust, or filtered exhaust. Additional groups were treated with DEN (4.5 mg/kg, s.c.) or B[a]P (20 doses of 0.25 mg itr. instillation)

and exposed to the three experimental atmospheres. No lung tumors were seen in the uninjected clean air group or in either diesel exhaust-exposed group. Initial treatment with DEN or B[a]P resulted in lung tumor incidences of only 10% and 2%, respectively, which were not significantly changed by exposure to diesel exhaust.

Heinrich et al. (1989b) reported results of experiments assessing the effects of DEN and diesel exhaust exposure in combination. Hamsters were exposed to exhaust from a Daimler-Benz 2.4-L engine operated at a constant load of about 15 kW and at a uniform speed of 2,000 rpm. The exhaust was diluted to an exhaust-clean air ratio of about 1:13, resulting in a mean particle concentration of 3.75 mg/m^3. The animals were exposed 19 h/day, 5 days/week beginning at noon each day, under a 12-h light cycle starting at 7 a.m. DEN (3 or 6 mg/kg) was given as a single s.c. injection 2 weeks from the start of exposure to groups of 40 male and 40 female Syrian golden hamsters exposed to whole diesel exhaust, filtered diesel exhaust, or clean air. Groups were also exposed to the exhaust without DEN or to only clean air. Exposures were conducted in chambers maintained at 22°C to 24°C and 40% to 60% relative humidity for up to 18 mo. Surviving hamsters were maintained in clean air for up to an additional 6 mo. The concentrations of B[a]P and B[e]P in the whole exhaust atmospheres were 37.5 and 61.9 ng/m^3, respectively.

No lung tumors were detected in any of the treatment groups. A nasal carcinoma was detected in a female hamster treated with DEN (6 mg/kg) and exposed to filtered exhaust. A tracheal carcinoma was detected in a male hamster exposed to whole diesel exhaust and receiving DEN (3 mg/kg), and a laryngeal carcinoma was observed in a male hamster receiving DEN (6 mg/kg) and exposed to whole diesel exhaust. Exposure of male hamsters to whole or filtered diesel exhaust alone did not result in a significant increase in the tumors relative to clean air controls. Male hamsters receiving 6 mg DEN/kg plus whole diesel exhaust exposure and dying before or after the 50% survival date, however, did show an increase in tumor rate compared with DEN-treated animals exposed to clean air. Using life-table analysis, a significant ($p<0.05$) exposure-related increase in tumor rate was noted for this group (40.0% vs. 7.0% for filtered exhaust + DEN and 7.0% for clean air + DEN). No upper respiratory tract tumors were detected in clean air controls or filtered-exhaust-exposed groups that did not receive the DEN treatment.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex. Diesel exhaust significantly enhanced the tumorigenic effects of DEN in males injected with 6 mg DEN/kg but not in females or in either sex given the 3 mg/kg dose. The co-carcinogenic effects of diesel with DEN therefore appear to be equivocal. It should be noted that the tumors occurred in the upper airways as opposed to the alveolar region in rats.

Brightwell et al. (1986, 1989) studied the effects of filtered or unfiltered diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females received no injections or s.c. injections of 4.5 mg DEN/kg 3 days before the start of exposure. The animals were 6 to 8 weeks old at the start of exposure to diesel exhaust at DPM concentrations of 0.7, 2.2, or 6.6 mg/m^3. They were exposed 16 h/day, 5 days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Appendix A. Although the DEN-pretreated hamsters exhibited an increase in tracheal papillomas in all treatment groups when compared with non-DEN pretreated hamsters, there was no statistically significant (*t* test) relationship between tumor incidence and exhaust exposure. As noted in IARC (1989), however, the reporting of tumor incidence and survival was incomplete.

7.2.4. Monkey Studies

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m^3) for 7 h/day, 5 days/week for 24 mo (Lewis et al., 1989). The same numbers of animals were also exposed to coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), diesel exhaust plus coal dust (1 mg/m^3 respirable concentration for each component; 3.2 mg/m^3 total concentration), or filtered air. Details of exposure conditions were listed previously in the description of the Lewis et al. (1986) study with rats (Appendix A).

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo timeframe employed in this study may not have allowed the manifestation of tumors in primates, because this duration is only a small fraction of the monkeys' expected lifespan. In fact, there have been no near-lifetime exposure studies in nonrodent species.

7.3. LUNG IMPLANTATION OR INTRATRACHEAL INSTILLATION STUDIES

7.3.1. Rat Studies

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that the PAHs in diesel exhaust that consist of four or more rings have a carcinogenic potential. Condensate was obtained from the whole exhaust of a 3.0 L passenger-car diesel engine connected to a dynamometer operated under simulated city traffic driving conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25% and 75% of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were surgically implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (B[a]P) controls were also included in the protocol (Table 7-2). Fraction IIb (made up of PAHs

Table 7-2. 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)	Number of carcinomas[a]	Number 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[c] 2 + 3 rings (IIa) (72)	19.22	103 (25-140)	0	1	0
PAH[d] 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.
[c]PAC = polycyclic aromatic compounds.
[d] PAH = polycyclic aromatic hydrocarbons.

Source: Adapted from Grimmer et al. (1987).

with four to seven rings), which accounted for only 0.8% of the total weight of DPM condensate, produced the highest incidence of lung carcinomas following implantation into the rat lungs. A carcinoma incidence of 17.1% 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% carcinoma incidence. Hydrophilic fractions of the DPM 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%) similar to the summed incidence of fraction IIb (17.1%) and IId (2.8%). The B[a]P positive controls (0.03, 0.1, 0.3 mg/rat) yielded a carcinoma incidence of 8.6%, 31.4%, and 77.1%, respectively. The study showed that the tumorigenic agents were primarily four- to seven-ring PAHs and, to a lesser extent, nitroaromatics. However, these studies demonstrated that simultaneous administration of various PAH compounds resulted in a varying of the tumorigenic effect, thereby implying that the tumorigenic potency of PAH mixtures may not depend on any one individual PAH. This study did not provide any information regarding the bioavailability of the particle-associated PAHs that might be responsible for carcinogenicity.

Kawabata et al. (1986) compared the effects of activated carbon and diesel exhaust on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with DPM 1 mg/week for 10 weeks). A second group of 31 rats was instilled with the same dosing regime of activated carbon. Twenty-seven rats received only the solvent (buffered saline with 0.05% Tween 80), and 53 rats were uninjected. Rats dying after 18 mo were autopsied. All animals surviving 30 mo or more postinstillation were sacrificed and evaluated for histopathology. Among 42 animals exposed to DPM surviving 18 mo or more, tumors were reported in 31, including 20 malignancies. In the subgroup surviving for 30 mo, tumors were detected in 19 of 20 animals, including 10 malignancies. Among the rats exposed to activated carbon, the incidence of lung tumors equaled 11 of 23 autopsied, with 7 cases of malignancy. Data for those dying between 18 and 30 mo and those sacrificed at 30 mo were not reported separately. Statistical analysis indicated that activated carbon induced a significant increase in lung tumor incidence compared with no tumors in 50 uninjected controls and 1 tumor in 23 solvent-injected controls. The tumor incidence increase was significant in the DPM-instilled group and was significantly greater than the increase in the carbon-instilled group. This study provides evidence for the carcinogenicity of DPM. It also shows, as Heinrich et al. (1995) and Nikula et al. (1995) did, that particles lacking organic constituents also can induce tumors.

Dasenbrock et al. (1996) conducted a study to determine the relative importance of the organic constituents of diesel particles and particle surface area in the induction of lung cancer in rats. Fifty-two female Wistar rats were intratracheally instilled with 16-17 doses of diesel

particles (DPM), extracted DPM, printex carbon black (PR), lampblack (LB), benzo[*a*]pyrene (B*a*P), DPM + B*a*P, or PR + B*a*P. The animals were held for a lifetime or sacrificed when moribund. The lungs were necropsied and examined for tumors. Diesel particles were collected from a Volkswagen 1.6 L engine operating on a US FTP-72 driving cycle. The mass median aerodynamic diameter (MMAD) of the diesel particles was 0.25 μm and the specific surface area was 12 m^2/gm. Following extraction with toluene MMAD increased to 138 m^2/gm. The MMAD for extracted PR was equal to 14 nm, while the surface area equaled 271 m^2/gm. The MMAD for extracted lampblack was equal to 95 nm, with a surface area equal to 20 m^2/gm. The B*a*P content of the treated particles was 11.3 mg per gm diesel particles and 29.5 mg B*a*P per gm PR. Significant increases in lung tumors were detected in rats instilled with 15 mg unextracted DPM and 30 mg extracted DPM, but not 15 mg extracted DPM. Printex CB was more potent than lamplack CB for induction of lung tumors, while B*a*P was effective only at high doses. Total dose and tumor responses are shown in Table 7-3.

A number of conclusions can be drawn from these results. First of all, particles devoid of organics are capable of inducing lung tumor formation, as indicated by positive results in the groups treated with high dose extracted diesel particles and printex. Nevertheless, extraction of organics from diesel particles results in a decrease in potency, indicating that the organic fraction does play a role in cancer induction. A relationship between cancer potency and particle surface area was also suggested by the finding that printex with a large specific surface area was more potent than either extracted DPM or lampblack, which have smaller specific areas. Finally, while very large doses of B*a*P are very effective in the induction of lung tumors, smaller doses adsorbed to particles surfaces had little detectable effect, suggesting that other organic components of DE may be of greater importance in the induction of lung tumors.

7.3.2. Syrian Hamster Studies

Kunitake et al. (1986) and Ishinishi et al. (1988b) conducted a study in which total doses of 1.5, 7.5, or 15 mg of a dichloromethane extract of DPM were instilled intratracheally over 15 weeks into male Syrian hamsters that were then held for their lifetimes. The tumor incidences of 2.3% (1/44), 0% (0/56), and 1.7% (1/59) for the high-, medium-, and low-dose groups, respectively, did not differ significantly from the 1.7% (1/56) reported for controls. Addition of 7.5 mg of B[a]P to a DPM extract dose of 1.5 mg resulted in a total tumor incidence of 91.2% and malignant tumor incidence of 88%. B[a]P (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2% (85% of these being malignant), which was not significantly different from the DPM extract + B[a]P group. Intratracheal administration of 0.03 μg B[a]P, the equivalent content in 15 mg of DPM extract, failed to cause a significant increase in tumors in

Table 7-3. Tumor incidences in rats following intratracheal instillation of diesel exhaust particles (DPM), extracted DPM, carbon black (CB), benzo[*a*]pyrene (B*a*P), or particles plus B*a*P

Experimental group	Number of animals	Total, dose	Animals with tumors (percent)	Statistical significance[a]
Control	47	4.5 mL	0 (0)	-
DPM (original)	48	15 mg	8 (17)	< 0.01
DPM (extracted)	48	30 mg	10 (21)	< 0.001
DPM (extracted)	48	15 mg	2 (4)	NS
CB (printex)	48	15 mg	10 (21)	< 0.001
CB (lampblack)	48	14 mg	4 (8)	NS
B*a*P	47	30 mg	43 (90)	< 0.001
B*a*P	48	15 mg	12 (25)	< 0.001
DEP + B*a*P	48	15 mg 170 µg B*a*P	4 (8)	NS
CB (printex) + B*a*P	48	15 mg 443 µg B*a*P	13 (27)	< 0.001

[a]Fischer's exact test.

rats. This study demonstrated a lack of detectable interaction between DPM extract and B[a]P, the failure of DPM extract to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of high doses of B[a]P. For studies using the DPM extract, some concern must be registered regarding the known differences in chemical composition between DPM extract and DPM. As with all intratracheal instillation protocols, DPM extract lacks the complement of volatile chemicals found in whole diesel exhaust.

The effects on hamsters of intratracheally instilled DPM suspension, DPM with Fe_2O_3, or DPM extract with Fe_2O_3 as the carrier were studied by Shefner et al. (1982). The DPM component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0 mg/week for 15 weeks to groups of 50 male Syrian golden hamsters. The total volume instilled was 3.0 mL (0.2 mL/week for 15 weeks). The DPM and dichloromethane extracts were suspended in physiological saline with gelatin (0.5% w/v), gum arabic (0.5% w/v), and propylene glycol (10% by volume). The Fe_2O_3 concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles (Fe_2O_3) without the DPM component. Two replicates of the experiments were performed. Adenomatous hyperplasia was reported to be most severe in those animals treated with DPM or DPM plus Fe_2O_3 particles and least severe in those animals receiving DPM plus Fe_2O_3. Of the two lung adenomas detected microscopically, one was in an animal treated with a high dose of DPM and the other was in an animal receiving a high dose of DPM extract. Although lung damage was increased by instillation of DPM, there was no evidence of tumorigenicity.

7.3.3. Mouse Studies

Ichinose et al. (1997a) intratracheally instilled 36 four-week-old male ICR mice per group weekly for 10 weeks with sterile saline or 0.05, 0.1, or 0.2 mg DPM. Particles were collected from a 2.74 L, four-cylinder Isuzu engine run at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of lung 8-hydroxydeoxyguanosine (8-OHdG). The remaining animals were sacrificed after 12 mo for histopathological analysis. Lung tumor incidence varied from 4/30 (13.3%) for controls to 9/30 (30%), 9/29 (31%), and 7/29 (24.1%) for mice instilled with 0.05, 0.1, and 0.2 mg/week, respectively. The increase in animals with lung tumors compared with controls was statistically significant for the 0.1 mg dose group, the only group analyzed statistically. Increases in 8-OHdG, an indicator of oxidative DNA damage, correlated well with increases in tumor incidences, with the correlation coefficients r = 0.916, 0.765, and 0.677 for the 0.05, 0.10, and 0.20 mg DPM groups, respectively.

In a similar study, 33 four-week-old male ICR mice per group were intratracheally instilled weekly for 10 weeks with sterile saline, 0.1 mg DPM, or 0.1 mg DPM from which the organic constituents were extracted with hexane (Ichinose et al., 1997b). Exhaust was collected from a 2.74 L four-cylinder Izuzu engine run at a steady speed of 2,000 rpm under a load of 6 torque (kg/m). Twenty-four hours after the last instillation, six animals per group were sacrificed for measurement of 8-OHdG. Surviving animals were sacrificed after 12 mo. The incidence of lung tumors increased from 3/27 (11.1%) among controls to 7/27 (25.9%) among those instilled with extracted diesel particles and 9/26 (34.6%) among those instilled with unextracted particles. The increase in number of tumor-bearing animals was statistically significant compared with controls ($p<0.05$) for the group treated with unextracted particles. The increase in 8-OHdG was highly correlated with lung tumor incidence, r = 0.99.

7.4. SUBCUTANEOUS AND INTRAPERITONEAL INJECTION STUDIES

7.4.1. Mouse Studies

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of i.p. injections of DPM on male Strong A mice. Three groups of 30 mice were injected with 0.1 mL of a suspension (particles in distilled water) containing 47, 117, or 235 μg of DPM collected from Fluoropore filters in the inhalation exposure chambers. The exposure system and exposure atmosphere are described in Appendix A. Vehicle controls received injections of particle suspension made up of 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 DPM dose of 1.1, 2.8, and 5.6 mg for the low-, medium-, and high-dose groups and 20 mg of urethan for the positive control group. These animals were sacrificed after 26 weeks and examined for lung tumors. For the low-, medium-, and high-dose DPM groups, the tumor incidence was 2/30, 10/30, and 8/30, respectively. The incidence among urethan-treated animals (positive controls) was 100% (29/29), with multiple tumors per animal. The tumor incidence for the DPM-treated animals did not differ significantly from that of vehicle controls (8/30) or negative controls (7/28). The number of tumors per mouse was also unaffected by treatment.

In further studies conducted by Orthoefer et al. (1981), an attempt was made to compare the potency of DPM with that of other environmental pollutants. Male and female Strain A mice were injected i.p. three times weekly for 8 weeks with DPM, DPM extracts, or various environmental mixtures of known carcinogenicity, including cigarette smoke condensate, coke oven emissions, and roofing tar emissions. Injection of urethan or dimethylsulfoxide (DMSO) served as positive or vehicle controls, respectively. In addition to DPM from the Nissan diesel

previously described, an 8-cylinder Oldsmobile engine operated at the equivalent of 40 mph was also used to compare emission effects from different makes and models of diesel engine. The mice were sacrificed at 9 mo of age and their lungs examined for histopathological changes. The only significant findings, other than for positive controls, were small increases in numbers of lung adenomas per mouse in male mice injected with Nissan DPM and in female mice injected with coke oven extract. Furthermore, the increase in the extract-treated mice was significant only in comparison with uninjected controls (not injected ones) and did not occur when the experiment was repeated. Despite the use of a strain of mouse known to be sensitive to tumor induction, the overall findings of this study were negative. The authors provided several possible explanations for these findings, the most likely of which were (1) the carcinogens that were present were very weak or (2) the concentrations of the active components reaching the lungs were insufficient to produce positive results.

Kunitake et al. (1986) conducted studies using DPM extract obtained from a 1983 HD MMC M-6D22P 11-L V-6 engine. Five s.c. injections of DPM extract (500 mg/kg per injection) resulted in a significant ($p<0.01$) increase in subcutaneous tumors for female C57BL mice (5/22 [22.7%] vs. 0/38 among controls). Five s.c. doses of DPM extract of 10, 25, 30, 100, or 200 mg/kg failed to produce a significant increase in tumor incidence. One of 12 female ICR mice (8.3%) and 4 of 12 male ICR mice (33.3%) developed malignant lymphomas following neonatal s.c. administration of 10 mg of DPM extract per mouse. The increase in malignant lymphoma incidence for the male mice was statistically significant at ($p<0.05$) compared with an incidence of 2/14 (14.3%) among controls. Treatment of either sex with 2.5 or 5 mg of DPM extract per mouse did not result in statistically significant increases in tumor incidence.

Additional studies using DPM extract from LD (1.8-L, 4-cylinder) as well as HD engines with female ICR and nude mice (BALB/c/cA/JCL-nu) were also reported (Kunitake et al., 1988). Groups of 30 ICR and nude mice each were given a single s.c. injection of 10 mg HD extract, 10 mg HD + 50 μg 12-O-tetradecanoylphorbol 13-acetate (TPA), 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) s.c. injection studies, Takemoto et al. (1988) presented additional data for subcutaneously administered DPM extract 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. DPM extract from HD or LD engines was administered weekly to the 5-week-old mice 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%], $p<0.01$), the 100 mg/kg LD group (6 of 32 [18.8%], $p<0.01$), and the 500 mg/kg LD group (7 of 32 [21.9%], $p<0.01$) in the adult mouse experiments. The tumors were characterized as malignant fibrous histiocytomas. No tumors were observed in other organs. The neonates were given single doses of 2.5, 5, or 10 mg DPM extract subcutaneously within 24 h of birth. There was a significantly higher incidence of malignant lymphomas in males receiving 10 mg of HD extract and of lung tumors for males given 2.5 mg HD extract and for males given 5 mg and females given 10 mg LD extract. A dose-related trend that was not significant was observed for the incidences of liver tumors for both the HD extract- and LD extract-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 was not dose related. The report concluded that LD DPM extract showed greater carcinogenicity than did HD DPM extract.

7.5. DERMAL STUDIES

7.5.1. Mouse Studies

In one of the earliest studies of diesel emissions, the effects of dermal application of extract from DPM were examined by Kotin et al. (1955). Acetone extracts were prepared from the DPM of a 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 C57BL and strain A mice. Results of these experiments are summarized in Table 7-4. In the initial experiments using 52 (12 male, 40 female) C57BL mice treated with DPM 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 DPM extract from an engine operated under full load were detected 16 mo after the start of treatment. Among female strain A mice treated with DPM 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%. Carcinomas as well as papillomas were seen, but the numbers were not reported.

Depass et al. (1982) examined the potential of DPM and dichloromethane extracts of DPM to act as complete carcinogens, carcinogen initiators, or carcinogen promoters. In skin-painting studies, the DPM was obtained from an Oldsmobile 5.7 L diesel engine operated under constant load at 65 km/h. The DPM was collected at a temperature of 100°C. Groups of 40 C3H/HeJ mice were used because of their low spontaneous tumor incidence. For the complete carcinogenesis experiments, DPM was applied as a 5% or 10% suspension in acetone. Dichloromethane extract was applied as 5%, 10%, 25%, or 50% suspensions. Negative controls

Table 7-4. Tumorigenic effects of dermal application of acetone extracts of diesel particulate matter (DPM)

Number of animals	Strain/sex	Sample material	Time to first tumor (mo)	Survivors at time of first tumor	Total tumors	Duration of experiment (mo)
52	C57BL/40 F C57BL/12 M	Extract of DPM obtained during warm-up	13	33	2	22
50	Strain A/M	Extract of DPM obtained during full load	15	8	4	23
25	Strain A/F	Extract of DPM obtained during full load	13	20	17	17

Source: Kotin et al. (1955).

received acetone, and positive controls received 0.2% B[a]P. For tumor-promotion experiments, a single application of 1.5% B[a]P was followed by repeated applications of 10% DPM suspension, 50% DPM extract, acetone only (vehicle control), 0.0001% phorbol 12-myristate 13-acetate (PMA) as a positive promoter control, or no treatment (negative control). For the tumor-initiation studies, a single initiating dose of 10% diesel particle suspension, 50% diesel particle extract, acetone, or PMA was followed by repeated applications of 0.0001% PMA. Following 8 mo of treatment, the PMA dose in the initiation and promotion studies was increased to 0.01%. 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-dose (50%) suspension of extract developed a squamous cell carcinoma after 714 days of treatment. Tumor incidence in the B[a]P group was 100%, and no tumors were observed in any of the other groups. For the promotion studies, squamous cell carcinomas with pulmonary metastases were identified in one mouse of the 50% DPM extract group and in one in the 25% extract group. Another mouse in the 25% extract group developed a grossly diagnosed papilloma. Nineteen positive control mice had tumors (11 papillomas, 8 carcinomas). No tumors were observed for any of the other treatment groups. For the initiation studies, three tumors (two papillomas and one carcinoma) were identified in the group receiving DPM suspension and three tumors (two papillomas and one fibrosarcoma) were found in the DPM extract group. These findings were reported to be statistically insignificant using the Breslow and Mantel-Cox tests.

The data from this study indicated that DPM 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) (Table 7-2), 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, DPM, and gasoline engine exhaust. DPM from five different engines, including a preproduction Nissan 220C, a 5.7-L Oldsmobile, a prototype Volkswagen Turbo Rabbit, a Mercedes 300D, and a HD Caterpillar 3304, was 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 DPM extracts were topically applied in single doses of 100, 500, 1,000 or 2,000 μg/mouse. The high dose (10,000 μg/mouse) was applied in

five daily doses of 2,000 μg. One week later, 2 μg of the tumor promoter tetradecanoylphorbol 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 Nissan engine DPM 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% for both groups. No significant carcinoma formation was noted for mice in the tumor-initiation experiments following exposure to DPM extracts of the other diesel engines, although the Oldsmobile engine DPM extract at 2.0 mg/mouse did produce a 40 percent papilloma incidence in male mice at 6 mo. This effect, however, was not dose dependent.

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 for complete carcinogenesis activity. DPM 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 7-5. The tumor initiation-promotion 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%. None of the DPM samples yielded positive results based on this criterion.

Kunitake et al. (1986, 1988) evaluated the effects of a dichloromethane extract of DPM obtained from a 1983 MMC M-6D22P 11-L V-6 engine. An acetone solution was applied in 10 doses every other day, followed by promotion with 2.5 μg of TPA three times weekly for 25 weeks. Exposure groups received a total dose of 0.5, 5, 15, or 45 mg of extract. Papillomas were reported in 2 of 50 animals examined in the 45 mg exposure group and in 1 of 48 in the 15 mg group compared with 0 of 50 among controls. Differences, however, were not statistically significant.

Table 7-5. Dermal tumorigenic and carcinogenic effects of various emission extracts

	Tumor initiation		Complete carcinogenesis	Tumor promotion
Sample	Papillomas[a]	Carcinomas[b]	Carcinomas[b]	Papillomas[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 mo.
[b]Cumulative score at 1 year.
[c]Male/female.
[d]ND = Not determined.
[e]I = Incomplete.

Source: Nesnow et al. (1982).

7.6. SUMMARY AND CONCLUSIONS OF LABORATORY ANIMAL CARCINOGENICITY STUDIES

As early as 1955, Kotin et al. (1955) provided evidence for tumorigenicity and carcinogenicity of acetone extracts of DPM following dermal application and also provided data suggesting a difference in this potential depending on engine operating mode. Until the early 1980s, no chronic studies assessing inhalation of diesel exhaust, the relevant mode for human exposure, had been reported. Since then, inhalation studies have been emphasized.

Studies using rats and an experimental protocol including long-term exposure at high exposure concentrations (up to 8 mg/m^3), resulting in large lung particle loads and a postexposure observation period, were generally positive in demonstrating DPM-induced increases in tumorigenicity. The highest incidences of tumors were reported by Brightwell et al. (1986, 1989). Among female rats exposed for 24 mo and held for their lifetimes, tumors were detected in 24 of 25 animals. This study points out the probable cumulative effects of high exposure concentration (6.6 mg/m^3), lengthy daily exposures (16 h/day), exposure in the dark resulting in a probable increase in ventilation and thereby DPM intake, and maintenance of the animals for their lifetimes. In two major studies reported by Heinrich et al. (1986a) and Mauderly et al. (1987), significant but lower lung tumor incidences were observed at the high-dose levels, 15.8% and 12.8%, respectively. Although exposure concentrations differed (7 mg/m^3 for Mauderly et al. vs. 4 mg/m^3 for Heinrich et al.), the longer daily exposure periods in the Heinrich et al. study, 19 h versus 7 h, would probably result in only slightly differing intakes. More recently, Nikula et al. (1995) provided additional data for rats, and Heinrich et al. (1995) reported on a study involving rats. In the Nikula et al. (1995) report, the percentages of rats (males and females combined) with neoplasms following exposure up to 23 mo were 1% (controls), 6% (2.5 mg/m^3 diesel exhaust), and 18% (6.5 mg/m^3 diesel exhaust). In the Heinrich et al. (1995) report, the percentages of rats with tumors following exposure up to 23 mo were <1% (controls), 0% (0.8 mg/m^3), 6% (2.5 mg/m^3), and 22% (7.0 mg/m^3).

Ishinishi et al. (1988a, 1988b) reported a 6.5% incidence of lung tumors in rats exposed to a concentration of 4 mg/m^3 DPM from a HD engine. In this study, although the concentration was relatively low, duration and length of daily exposure were long (16 h/day for 30 mo). Iwai et al. (1986) reported an increased lung tumor incidence (4/14) in Fischer rats exposed 8 h/day, 7 days/week for 24 mo to a DPM concentration of 4.9 mg/m^3. Four of five rats held in clean air for an additional 3 to 6 mo, however, also developed tumors, pointing out again the importance of a long study duration. Iwai et al. (1986) reported the only diesel exhaust inhalation-induced tumor increase at a nonrespiratory site (splenic lymphoma).

Low exposure concentrations and/or short exposure durations were generally used in the negative studies (Karagianes et al. 1981; Lewis et al. 1989; White et al. 1983; Takemoto et al., 1986). The lowest DPM concentrations resulting in significant positive effects in rats were in the range of 2 to 3 mg/m^3.

Inhalation of diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986a; Stöber, 1986) and in female Sencar mice (Pepelko and Peirano, 1983). An apparent increase was also seen in female C57BL mice (Takemoto et al., 1986). In a series of inhalation studies using strain A mice, no increases in lung tumor rates were detected (Orthoefer et al., 1981; Kaplan et al., 1982; Kaplan et al., 1983; White et al., 1983). The only study in which lung tumor incidences were increased in animals exposed to filtered exhaust was reported by Heinrich et al. (1986a) and Stöber (1986) using NMRI mice. In a more recent study by Heinrich et al. (1995) exposure of NMRI and C57BL/6N mice to diesel exhaust (4.5 mg/m^3 for up to 23 mo) did not produce a tumorigenic response that was significantly different from that observed in clean air controls.

Attempts to induce significant increases in lung tumors in Syrian hamsters were unsuccessful after inhalation (Heinrich et al., 1982; Heinrich et al., 1986a; Heinrich et al., 1989b; Brightwell et al., 1986) or itr. instillation (Kunitake et al., 1986; Ishinishi et al., 1988b). Neither cats (Pepelko and Peirano, 1983 [see Chapter 4]) nor monkeys (Lewis et al., 1986) developed tumors following 2-year exposure to diesel exhaust. The duration of these exposures, however, may well have been inadequate in these two longer-lived species. Exposure levels were also below the maximum tolerated dose (MTD) in the monkey studies and borderline for detection of lung tumor increases in rats.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of DPM. Grimmer et al. (1987) showed not only that an extract of DPM was carcinogenic when instilled in the lungs of rats but also that most of the carcinogenicity resided in the portion containing PAHs with four to seven rings.

Alternative exposure routes including dermal exposure and s.c. injection in mice provided additional evidence for tumorigenic effects of DPM. Particle extracts applied dermally to mice have been shown to induce significant skin tumor increases in two studies (Kotin et al., 1955; Nesnow et al., 1982). Kunitake et al. (1986) also reported a marginally significant increase in skin papillomas in ICR mice treated with an organic extract from an HD diesel engine. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of DPM 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. A significant increase in the incidence of sarcomas in female C57Bl mice was reported by Kunitake et al. (1986) following s.c. administration of LD DPM extract at doses of 500 mg/kg. Takemoto et al. (1988) provided additional data for this study and reported an increased tumor incidence in the mice following injection of LD engine DPM extract at doses of 100 and 500 mg/kg. Results of i.p. injection of DPM or DPM extracts in strain A mice were generally negative (Orthoefer et al., 1981; Pepelko and Peirano, 1983), suggesting that the strain A mouse may not be a good model for testing diesel emissions.

Experiments using tumor initiators such as DEN, B[a]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986a; Takemoto et al., 1986) did not provide conclusive results regarding the tumor-promoting potential of either filtered or whole diesel exhaust. A report by Heinrich et al. (1982), however, indicated that filtered exhaust may promote the tumor-initiating effects of DEN in hamsters.

Several reports (Wong et al., 1986; Bond et al., 1990) affirm observations of the potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. These findings are discussed in more detail in Chapter 9. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 8.

It appears reasonably certain that with adequate exposures, inhalation of diesel exhaust will induce lung cancer in rats. The relationship between exposure levels and response, however, is less clearcut. Although significant increases in lung tumors were not reported at concentrations less than about 2 mg/m^3, the response at higher concentrations varies considerably. A significant percentage of this variation can probably be attributed to the exposure regime. A better method than concentration alone for assessing exposure-response relationships could be achieved by comparing cumulative exposure (concentration × daily exposure duration × days of exposure). Only those studies conducted for a sufficient length of time (≥24 mo) for expression of carcinogenic responses have been included in this analysis. Examination of the rat data (shown in Table 7-6 and plotted in Figure 7-1) reveals that most studies indicate a trend of increasing tumor incidence at exposures exceeding 1×10^4 $mg \cdot hr/m^3$.

A similar comparison could not be adequately made for mice, because experimental designs were not comparable. The study reported by Stöber (1986), for example, involved lifetime exposure following weaning. In the studies reported by Pepelko and Peirano (1986), however, Sencar mice were exposed from conception, when they are presumably more sensitive to tumor induction, and sacrificed at 15 mo, whereas strain A mice were sacrificed at 9 mo of age because of the rapid increase in the incidence of lung tumors in controls. The successful

Table 7-6. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust

Study	Exposure rate/duration (h/week, mo)	Total exposure time (h)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot h/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Mauderly et al. (1987)	35, 30	4,200	0	0	0	0.9
	35, 30	4,200	0.35	12.25	1,470	1.3
	35, 30	4,200	3.5	122.5	14,700	3.6
	35, 30	4,200	7.1	248.5	29,820	12.8
Nikula et al. (1995)	80, 23	7,360	0	0	0	1.0
	80, 23	7,360	2.5	200.0	18,400	7.0
	80, 23	7,360	6.5	520.0	47,840	18.0
Heinrich et al. (1986b)	95, 35	13,300	0	0	0	0
	95, 35	13,300	4.24	402.8	56,392	17.8
Heinrich et al. (1995)	90, 24	8,640	0	0	0	0
	90, 24	8,640	0.8	72.0	7,400[b]	0
	90, 24	8,640	2.5	225.0	21,800[b]	5.5
	90, 24	8,640	7.0	630.0	61,700[b]	22.0
Ishinishi et al. (1988a)	96, 30	11,520	0	0	0	3.3
	96, 30	11,520	0.1	9.6	1,152	2.4
(Light-duty engine)	96, 30	11,520	0.4	38.4	4,608	0.8
	96, 30	11,520	1.1	105.6	12,672	4.1
	96, 30	11,520	2.3	220.8	26,496	2.4
(Heavy-duty engine)	96, 30	11,520	0	0	0	0.8
	96, 30	11,520	0.5	48.0	5,760	0.8
	96, 30	11,520	1.0	96.0	11,520	0
	96, 30	11,520	1.8	172.8	20,736	3.3
	96, 30	11,520	3.7	355.2	42,624	6.5

Table 7-6. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust (continued)

Study	Exposure rate/duration (h/week, mo)	Total exposure time (h)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot h/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Brightwell et al. (1989)	80, 24	7,680	0	0	0	1.2
	80, 24	7,680	0.7	56.0	5,376	0.7
	80, 24	7,680	2.2	176.0	16,896	9.7
	80, 24	7,680	6.6	528.0	50,688	38.5
Kaplan et al. (1983)	140, 15	8,400	0	0	0	0
	140, 15	8,400	0.25	35.0	2,100	3.3
	140, 15	8,400	0.75	105.0	6,300	10.0
	140, 15	8,400	1.5	210.0	12,600	3.3
Iwai et al. (1986)	56, 24	5,376	0	0	0	0
	56, 24	5,376	4.9	274.4	26,342	36.8
Takemoto et al. (1986)	16, 18-24	1,152-1,536	0	0	0	0
	16, 18-24	1,152-1,536	2-4	32-64	3,456-4,608	0
Karagianes et al. (1981)	30, 20	2,400	0	0	0	0
	30, 20	2,400	8.3	249	19,920	16.6

[a]Combined data for males and females.
[b]As reported in Heinrich et al. (1995).

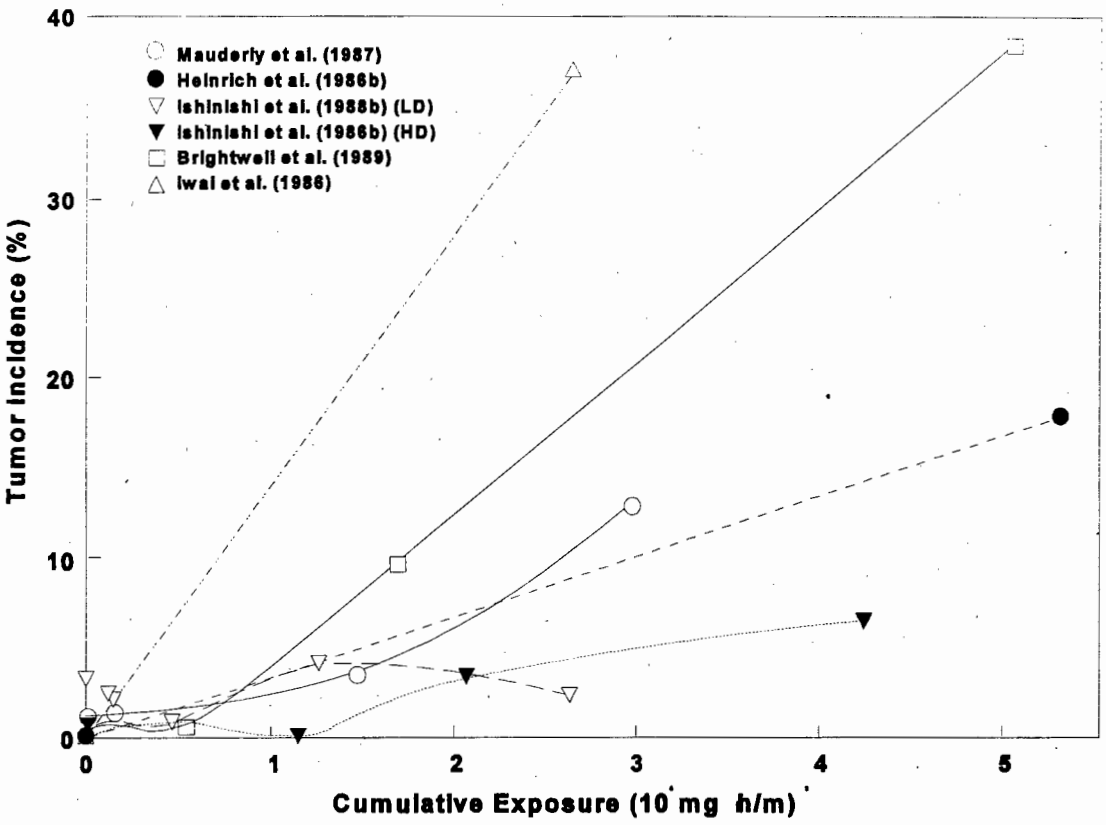

Figure 7-1. Cumulative exposure data for rats exposed to whole diesel exhaust.

induction of lung tumors in mice by Ichinose et al. (1997a,b) via intratracheal instillation may be the result of focal deposition of larger doses.

Although the preceding analysis accounts for total inspired dose, it does not account for delayed particle clearance at higher exposure levels. The analysis might be improved further by comparing tumor response with lung burden of particulate matter. This analysis could account for not only differences in exposure times but also for overload inhibition of clearance at high exposure levels. Although such data were not available for all studies, it could be estimated by using available estimates of respiration along with deposition and clearance models. The extrapolation models (Appendix A) and the qualitative/quantitative evaluations of Chapter 11 attempt this relative to human exposure.

To evaluate accurately the carcinogenic risk to humans from diesel engine emissions, it is important to ascertain the fraction or fractions of exhaust responsible for inducing lung tumors. Several of the previously discussed studies indicated that only whole (unfiltered) diesel exhaust is tumorigenic or carcinogenic and that these responses are eliminated or greatly minimized in exposures to filtered diesel exhaust. Data for NMRI and C57BL/6N mice exposed to whole diesel exhaust (4.5 m/m^3), filtered exhaust, or clean air for up to 23 mo showed no significant

increases in tumor incidences for mice exposed to filtered exhaust relative to clean air controls (Heinrich et al., 1995). In one study (Stöber, 1986), however, a significant increase in lung tumors was seen in mice exposed to filtered exhaust. Heinrich et al. (1982) also provided some evidence suggesting that the gaseous fraction promoted the tumorigenic effects of DEN. Nevertheless, because of the lack of positive data in rats and the limited positive data in mice, the tumorigenicity of the gaseous fraction must be considered to be unresolved.

The importance of DPM has been affirmed for the tumorigenic response observed in rats (Heinrich et al., 1995; Nikula et al., 1995). Evidence for the importance of the carbon core was initially provided by studies of Kawabata et al. (1986), which showed induction of lung tumors following intratracheal instillation of carbon black that contained no more than traces of organics and studies of Heinrich (1990) that indicated that exposure via inhalation to carbon black (Printex 90) particles induced lung tumors at concentrations similar to those effective in DPM studies. Induction of lung tumor by other particles of low solubility such as titanium dioxide (Lee et al., 1986) confirmed the capability of particles in inducing lung tumors. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having much higher PAH concentrations than DPM, also was effective in tumor induction (Heinrich et al., 1986b; 1994).

The relative importance of the adsorbed organics, however, remains to be elucidated and is of some concern because of the known carcinogenic capacity of some of these chemicals. These include polycyclic aromatics as well as nitroaromatics as described in Chapter 2. Organic extracts of particles also have been shown to induce tumors in a variety of injection, intratracheal instillation, and skin-painting studies, and Grimmer et al. (1987) have, in fact, shown that the great majority of the carcinogenic potential following instillation resided in the fraction containing four- to seven-ring PAHs.

In summary, based on positive inhalation exposure data in rats and mice, intratracheal instillation in rats, and injection or skin painting in mice and supported by positive mutagenicity studies, the evidence for carcinogenicity of diesel exhaust is considered to be adequate. The contribution of the various fractions of diesel exhaust to the carcinogenic response is less certain. The effects of the gaseous phase are equivocal. The presence of known carcinogens adsorbed to diesel particles and the demonstrated tumorigenicity of particle extracts in a variety of injection, instillation, and skin-painting studies suggest carcinogenic potential for the organic fraction. Studies showing that insoluble particles (e.g., carbon black, TiO_2) can also induce tumors, on the other hand, have provided definitive evidence that the carbon core of the diesel particle is instrumental in the carcinogenic response observed in rats.

A summary of studies assessing the tumorigenic and carcinogenic effects in laboratory animals following inhalation exposure to diesel exhaust is presented in Table 7-1.

7.7. REFERENCES

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8. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EMISSIONS

8.1. INTRODUCTION

Emissions from diesel engine 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 adsorbed on the surface. Diesel engine exhaust contains about 100 times more particulate matter than gasoline engine exhaust.

In this chapter, various mortality and morbidity studies of the health effects of exposure to diesel engine emissions are reviewed. Although an attempt was made to cover all the relevant studies, a number of studies are not included for several reasons. First, the change from steam to diesel engines in locomotives began in 1935 and was about 95% complete by 1959 (Garshick et al., 1988). Diesel buses also were introduced about the same time. Therefore, exposure to diesel exhaust was less common, and the follow-up period for studies conducted prior to 1959 (Raffle, 1957; Kaplan, 1959) was not long enough to cover the long latency period of lung cancer. The usefulness of these studies in evaluating the carcinogenicity of diesel exhaust is greatly reduced; thus, they are not considered here.

Second, hypothesis-generating studies were excluded from this review because their findings need subsequent confirmation by definitive studies (Silverman et al., 1983; Schenker et al., 1984; Buiatti et al., 1985; Flodin et al., 1987; Siemiatycki et al., 1988; Swanson et al., 1993; Cordier et al., 1993; Notani et al., 1993).

Third, studies in which exposure to diesel exhaust was uncertain or was defined as motor exhaust (which includes both gasoline and diesel exhaust) were excluded because they would have contributed little to the evaluation of the carcinogenicity of diesel exhaust (Waxweiler et al., 1973; Ahlberg et al., 1981; Stern et al., 1981; Vineis and Magnani, 1985; Gustafsson et al., 1986; Silverman et al., 1986; Jensen et al., 1987; Garland et al., 1988; Risch et al., 1988; Guberan et al., 1992).

Fourth, a study by Coggon et al. (1984) was not included because the occupational information abstracted from death certificates had not been validated; this would have resulted in limited information.

Three types of studies of the health effects of exposure to diesel engine emissions are reviewed in this chapter: (1) cohort studies, (2) case-control studies of lung cancer, and (3) case-control studies of bladder cancer. In the cohort studies, the cohorts of heavy construction equipment operators, railroad and locomotive workers, and bus garage employees were studied

retrospectively to determine increased mortality and morbidity resulting from exposures to varying levels of diesel emissions in the workplace. A total of nine cohort mortality (one of the mortality studies also included a nested lung cancer case-control study), ten lung cancer case-control, and seven bladder cancer case-control studies are considered in this section.

8.2. COHORT STUDIES

8.2.1. Waller (1981): Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes

A retrospective 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. Nearly 20,000 male employees aged 45 to 64 were followed for the 25-year period between 1950 and 1974, constituting a total of 420,700 man-years at risk. These were distributed among five job categories: drivers, garage engineers, conductors, motormen or guards, and engineers (works). Most employees 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-year periods and 5-year age ranges, finally combining the results to obtain the total expected deaths in the required age range for the calendar period. A total of 667 cases of lung cancer was reported, compared with 849 expected, to give a mortality ratio of 79%. In each of the five job categories, the observed numbers were below those expected. Engineers in garages had the highest mortality ratio (90%), but this did not differ significantly from the other job categories. Environmental sampling was done at one garage, on 1 day in 1979, 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 occurred while the worker was employed (the 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, information on resignees who may have had significant exposure to diesel exhaust, and lung cancer deaths among them, was not available for analysis. This fact may have led to a dilution effect, resulting in underascertainment 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 the 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 (45 to 64 years of age) 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 the 25-year follow-up period. 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 its earlier years was exposed to much higher concentrations of environmental contaminants than currently exist. It is not clear when the reduction in benzo[a]pyrene concentration occurred because there are no environmental readings available between 1957 and 1979. It is also important to note that the concentrations of benzo[*a*]pyrene inside the garage in 1957 were not very different from those outside the garage, thus indicating that exposure for garage workers was not much different from that of the general population. Last, no data were collected on smoking habits.

8.2.2. Howe et al. (1983): Cancer Mortality (1965 to 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 that is regularly updated and used by the company for payment of pensions. To receive a pension, each pensioner must provide, on a yearly basis, evidence 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%) were successfully linked to a record in the mortality file. A random sample manual check on unlinked data revealed that failure to link was due mainly 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: (1) nonexposed, (2) possibly exposed, and (3) probably exposed. Person-years of observation were calculated and classified by age at observation in 5-year age groups (35 to 39, 40 to 44, . . ., 80 to 84, and ≥85 years). 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 (SMRs) were then calculated using rates of the Canadian population for the period between 1965 and 1977.

Both total mortality (SMR = 95, $p<0.001$) and all cancer deaths (SMR = 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 ($p=0.013$); and, 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 ($p<0.001$). Analysis performed after the exclusion of individuals who worked in the maintenance of steam engines, and hence were exposed to high levels of asbestos, yielded the risk of lung cancer to be 1.00, 1.21, and 1.33 for those nonexposed, possibly exposed, and probably exposed to diesel exhaust, respectively, with a highly significant trend ($p<0.001$).

An analysis done on individuals who retired prior to 1950 showed the relative risk of lung cancer among nonexposed, possibly exposed, and probably exposed to be 1.00, 0.70, and 0.44, respectively, based on fewer than 15 deatns in each category. A similar analysis of individuals who retired after 1950 found the results in the same categories to be 1.00, 1.23, and 1.40, respectively. Although retirement prior to 1950 indicated exposure to coal dust alone, retirement after 1950 shows the results of mixed exposure to coal dust and diesel fumes. As there was considerable overlap between occupations involving probable exposure to diesel fumes and probable exposure to coal dust, and as 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 or a mixture of both.

Although this study showed a highly significant dose-response relationship between diesel fumes and lung cancer, it has some methodological limitations. There were concurrent exposures to both diesel fumes and coal dust during the transition period; therefore, misclassification of exposure may have occurred, because only occupation at retirement was available for analysis. It is possible that the elevated response observed for lung cancer was due to the combined effects of exposure to both coal dust and diesel fumes and not 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 of coal miners. 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 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.

8.2.3. Rushton et al. (1983): Epidemiological Survey of Maintenance Workers in the London Transport Executive Bus Garages and Chiswick Works

This is a retrospective mortality cohort study of male maintenance workers employed for at least 1 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. One hundred different job titles were coded into 20 broader groups. These 20 groups were not ranked for diesel exhaust exposure, though. The reason for leaving was coded as died in service, retired, or other. The underlying cause of death was coded using the eighth revision of the International Classification of Diseases (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-year age and calendar period groups. The expected number of deaths was calculated by applying the 5-year 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.

The number of person-years of observation totaled 50,008 and was contributed by 8,490 individuals in the study with a mean follow-up of 5.9 years. Only 2.2% (194) of the men were not traced. Observed deaths from all causes were significantly lower than expected (observed = 495, $p<0.001$). 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. All the excess deaths observed for the various job groups, except for the general hand category, were based on very small numbers (usually smaller than five) and merited cautious interpretation. Only a notable excess in the general hand category for lung cancer was based on 48 cases (SMR = 133, $p<0.03$). However, given the fact that there was no adjustment for confounding variables such as smoking, the result should be interpreted cautiously.

This mortality study of London transport maintenance workers did not demonstrate any cancer excesses based on a large number of cases; this needs further exploration. Its limitations, including the small sample size, short duration of follow-up (average of only 6 years), and lack of sufficient latency period, make this study inadequate to draw any conclusions. 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 exposure. Death information was ascertained from death certificates, with inherent problems of inaccuracy, misdiagnosis, and errors in coding, and it was not known whether a trained nosologist coded the death certificates. No adjustments were made for the confounding effects of smoking and socioeconomic factors.

8.2.4. Wong et al. (1985): Mortality Among Members of a Heavy Construction Equipment Operators Union With Potential Exposure to Diesel Exhaust Emissions

This is a retrospective 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, CA. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 year 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. Approximately 50% of the cohort had been union members for less than 15 years, whereas the other 50% had been union members for 15 years or more. The average duration of membership was 15 years. As of December 31, 1978, 29,046 (85%) cohort members were alive, 3,345 (9.8%) were dead, and 1,765 (5.2%) remained untraced. Vital status of 10,505 members who had left the union as of December 31, 1978, was ascertained from the Social Security Administration. Death certificates were obtained from appropriate state health departments. Altogether, 3,243 deaths (for whom death certificates were

available) in the cohort were coded using the seventh revision of the ICD. For 102 individuals, death certificates could not be obtained, only the date of death; these individuals were included in the calculation of the SMR for all causes of death but were deleted from the cause-specific SMR analyses. Expected deaths and SMRs were calculated using the U.S. national age-sex-race cause-specific mortality rates for 5-year time periods between 1964 and 1978. The entire cohort population contributed to 372,525.6 person-years in this 5-year study period.

A total of 3,345 deaths was observed, compared with 4,109 expected. The corresponding SMR for all causes was 81.4 (p=0.01), which confirmed the "healthy worker effect." A total of 817 deaths was attributed to malignant neoplasms, slightly fewer than the 878.34 expected based on 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 SMR was observed for cancer of the liver (SMR = 166.7, observed = 23, p<0.05).

Analysis by length of union membership as a surrogate of duration for potential exposure showed statistically significant increases in SMRs of cancer of the liver (SMR = 424, p<0.01) in the 10- to 14-year membership group and of the stomach (SMR = 248, p<0.05) in the 5- to 9-year membership group. No cancer excesses were observed in the 15- to 19-year and 20+-year membership groups. Although the SMR for cancer of the lung had a statistically significant deficit in the less than 5-year duration group, it showed a positive trend with increasing length of membership, which leveled off after 10 to 14 years.

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 a statistically significant SMR excess for cancer of the liver (10- to 19-year group, SMR = 257.9). The SMR for cancer of the lung showed a statistically significant deficit for a <10-year latency 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, 6,678 individuals contributing to 32,670.1 person-years, showed statistically significant increases (p<0.01) in SMRs for all cancers; all causes of death; cancers of the digestive system, large intestine, respiratory system, and lung; emphysema; and cirrhosis of the liver. The other two significant excesses (p<0.01) were for lymphosarcoma and reticulosarcoma and nonmalignant respiratory diseases. Further analysis of the 4,075 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 (p<0.05) for all cancers, cancer of the lung, and lymphosarcoma and reticulosarcoma.

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 cancer of the lung among bulldozer operators who had 15 to 19 years of membership and 20+ years of follow-up (SMR = 343.4, $p<0.05$). This excess was based on 5 out of 495 deaths observed in this group of 6,712 individuals, who contributed 80,327.6 person-years of observation.

The cause-specific mortality analysis in the low-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 years of membership and length of follow-up of at least 20 years, and cancer of the liver (SMR = 871.9, observed = 3, $p<0.05$) among those with 10 to 14 years of membership and length of follow-up of 10 to 19 years. There were 7,032 individuals who contributed to 78,402.9 person-years of observation in the low-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% 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, $p<0.01$).

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.

Cancer of the lung 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, because workers without job histories included those who had the same job before and after 1967 and thus may have worked 12 to 14 years or longer. If they had belonged to the category in which 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 because such information may be quite informative with regard to the evaluation of the carcinogenicity of diesel exhaust.

The study design is adequate, covers about a 15-year observation period, has a large enough population, and is appropriately analyzed; however, it has too many limitations to permit any conclusions. First, no exposure histories are available. One has to make do with 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 is included in the same category; this would have a dilution effect, since extremely variable exposures were considered in the study. Second, the length of time worked in any particular job is not available. Third, work histories were not available for 9,707 individuals, who contributed 104,447.5 person-years, a large proportion of the study cohort (28%). 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 was not ruled out. Last, although 34,156 members were eligible for the study, the vital status of 1,765 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 being underestimated.

As for the smoking survey, the investigators took a very small sample (133 out of 34,156, which was not even 1%). Of 133, only 107 (80%) participated. It was a systematic sample, but the authors neglected to mention how the list was prepared. Hence, the sample may not be representative of the study population and, with a small sample size, the results are not generalizable. The questionnaire asked only for current smoking history. No detailed history was obtained for the amount smoked or length of smoking history, both of which have a bearing on emphysema as well as lung carcinoma.

8.2.5. Edling et al. (1987): Mortality Among Personnel Exposed to Diesel Exhaust

This is a retrospective cohort mortality 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 at five different bus companies in southeastern Sweden between 1950 and 1959. Based on 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-year 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 total cohort of 694 men (after loss of 5 men to follow-up) was divided into three exposure categories: (1) clerks with the lowest exposure, (2) bus drivers with moderate exposure, and (3) bus garage workers with highest exposure.

The 694 men provided 20,304 person-years of observation, with 195 deaths compared with 237 expected. A deficit in cancer deaths largely accounted for this lower-than-expected mortality in the 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-year latency period and either any exposure or an exposure exceeding 10 years, similar results were obtained, with fewer neoplasms than expected, whereas cardiovascular diseases showed risk around or slightly above unity.

Five lung cancer deaths were observed among bus drivers who had moderate diesel exhaust exposure, whereas 7.2 were expected. The only other lung cancer death was observed among bus garage workers who had the highest diesel exhaust exposure. The small size of the cohort and poor data on diesel exhaust exposure are among the major limitations of this study. Although lifetime occupational histories were 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% 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.

8.2.6. 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 years of follow-up. The analysis was restricted to males aged 40 to 79 years 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 and to employment in selected occupations related to diesel exhaust exposure. In 1982, more than 77,000 American Cancer Society volunteers enrolled over 1.2 million 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 years 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. The questionnaire also included three questions on occupation: (1) current occupation, (2) last occupation, if retired, and (3) 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 other year during the study. Death certificates were then obtained from state health departments and coded according to a system based on the ninth revision of the ICD 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 in relation to 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 person-years of risk were available for analysis. Three

percent of the subjects (14,667) had not given any smoking history, and 20% (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 diesel exhaust exposure with the excluded population with no information on diesel exhaust exposure showed that the mean ages were 54.7 and 57.7 years, the nonsmokers were 72.4% and 73.2%, and the total mortality rates per 1,000 per year were 23.0% and 28.8%, respectively.

The all-cause mortality was elevated among railroad workers (relative risk [RR] = 1.43, 95% confidence interval [CI] = 1.2, 1.72), heavy equipment operators (RR = 1.7, 95% CI = 1.19, 2.44), miners (RR = 1.34, 95% CI = 1.06, 1.68), and truck drivers (RR = 1.19, 95% CI = 1.07, 1.31). For lung cancer mortality the risks were significantly elevated for miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.60, 95% CI = 1.12, 6.06). Risks were also elevated but not significantly for railroad workers (RR = 1.59, 95% CI = 0.94, 2.69) and truck drivers (RR = 1.24, 95% CI = 0.93, 1.66). These risks were calculated according to the Mantel-Haenszel method, controlling for age and smoking. Although the 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 years, the relative risk was 0.87, while for drivers who worked for more than 16 years, the relative risk was 1.33 (95% 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 composed 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% 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. Although only 0.8% 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 diesel exhaust exposure occupations, such

as heavy equipment operators, truck drivers, and railroad workers, showed elevated lung cancer risk, this study is suggestive of a causal association.

8.2.7. 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% CI = 1.06, 1.88) for lung cancer with 20 years of work in jobs with diesel exhaust exposure. To confirm these results, a large retrospective cohort mortality 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% 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 where workers were asked to indicate the amount of time spent in railroad locations, either near or away from sources of diesel exhaust, was used to validate this classification. Workers selected for this survey were actively employed at the time of the survey, 40 to 64 years of age, who started work between 1939 and 1949, in the job codes sampled in 1959, and were eligible for railroad benefits. To qualify for benefits, a worker must have 10 years or more of service with the railroad and should not have worked for more than 2 years in a nonrailroad job after leaving railroad work. Workers with recognized asbestos exposure, such as repair of asbestos-insulated steam locomotive boilers, passenger cars, and steam pipes, or railroad building construction and repairs, were excluded from 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 both ways, with and without them.

The death certificates for 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 departments of health. Coding of cause of death was done without knowledge of exposure history, according to the eighth revision of the ICD. 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% 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-year age group in 1959. The standardized rates were based on the overall 5-year person-year time distribution of individuals in each age group starting in 1959. The only exception to this was between 1979 and 1980, when a 2-year person-year distribution was used. The Mantel-Haenszel analogue for person-year data was used to calculate 95% confidence intervals for the standardized rate ratios.

The cohort consisted of 55,407 workers, 19,396 of whom had died by the end of 1980. Death certificates were not available for 11.7% of all deaths. Of the 17,120 deaths for whom death certificates were obtained, 48.4% were attributable to diseases of the circulatory system, whereas 21% were attributable to all neoplasms. Of all neoplasms, 8.7% (1,694 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 proportion 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% CI = 1.11, 1.89) among the age group 40 to 44 years and a relative risk of 1.33 (95% CI = 1.03, 1.73) for the age group 45 to 49 years. Risk estimates in the older age groups 50 to 54, 55 to 59, and 60 to 64 years 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 relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) in the 1959 age groups 40 to 44 years and 45 to 49 years. Directly standardized rate ratios were also calculated for each 1959 age group based on diesel exhaust 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 years prior to death, the risk of dying from

lung cancer increased with the number of years worked in a diesel-exhaust-exposed job. In this analysis, exposure to diesel exhaust was analyzed by exposure duration groups and in a model entering age in 1959 as a continuous variable. The workers with greater than 15 years of exposure had a relative risk of lung cancer of 1.72 (95% CI = 1.27, 2.33). The risk for 1 to 4 years of cumulative exposure was 1.20 (95% CI = 1.01, 1.44); for 5 to 9 years of cumulative exposure, it was 1.24 (95% CI = 1.06, 1.44); and for 10 to 14 years of cumulative exposure, it was 1.32 (95% 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 confirmed 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 years of age in 1959 classified as diesel exhaust-exposed, 94% of the cases were still in diesel exhaust-exposed jobs 20 years later.

The main limitation of the study is the lack of quantitative data on exposure to diesel exhaust. This is one of the few studies in which industrial hygiene measurements of diesel exhaust were done. These measurements were correlated with job titles to divide the cohort in dichotomous exposure groups of exposed and nonexposed. This may have led to an underestimation of the risk of lung cancer since exposed groups included individuals with low to high exposure. The number of years exposed to diesel exhaust was used as a surrogate for dose. The dose, based on duration of employment, may have been inaccurate because individuals were working on steam or diesel locomotives during the transition period. If the categories of exposure to diesel exhaust would have been set up as no, low, moderate, and high exposure, the results would have been more meaningful and so would have been the dose-response relationship. Another limitation of this study was the inability to examine the effect of years of exposure and latency. No adjustment for smoking was made in this study. However, an earlier case-control study done in the same cohort (Garshick et al., 1987) showed no significant difference in the risk estimate after adjusting for smoking. Despite these limitations, the results of this study demonstrate that occupational exposure to diesel exhaust is associated with a modest risk (1.5) of lung cancer.

8.2.8. Gustavsson et al. (1990): Lung Cancer and Exposure to Diesel Exhaust Among Bus Garage Workers

A retrospective mortality study (from 1952 to 1986), cancer incidence study (from 1958 to 1984), and nested case-control study were conducted among a cohort of 708 male workers from five bus garages in Stockholm, Sweden, who had worked for at least 6 months between 1945 and 1970. Thirteen individuals were lost to follow-up, reducing the cohort to 695.

Information was available on location of workplace, job type, and beginning and ending of work periods. Workers were traced using a computerized register of the living population, death and burial books, and data from the Stockholm city archives.

For the cohort mortality analyses, death rates of the general population of greater Stockholm were used. Death rates of occupationally active individuals, a subset of the general population of greater Stockholm, were used as a second comparison group to reduce the bias from "healthy worker effect." Mortality analysis was conducted using the "occupational mortality analysis program" (OCMAP-PC). For cancer incidence analysis, the "epidemiology in Linköping" (EPILIN) program was used, with the incidence rates obtained from the cancer registry.

For the nested case-control study, both dead and incident primary lung cancers, identified in the register of cause of deaths and the cancer register, were selected as cases (20). Six controls matched on age ±2 years, selected from the noncases at the time of the diagnosis of cases, were drawn at random without replacements. Matched analyses were done to calculate odds ratios using conditional logistic regression. The EGRET and Epilog programs were used for these analyses.

Diesel exhaust and asbestos exposure assessments were performed by industrial hygienists based on the intensity of exposure to diesel exhaust and asbestos, specific for workplace, work task, and calendar time period. A diesel exhaust exposure assessment was based on (1) amount of emission (number of buses, engine size, running time, and type of fuel), (2) ventilatory equipment and air volume of the garages, and (3) job types and work practices. Based on detailed historical data and very few actual measurements, relative exposures were estimated (these were not absolute exposure levels). The scale was set to 0 for unexposed and 1 for lowest exposure, with each additional unit increase corresponding to a 50% increase in successive intensity (i.e., 1.5, 2.25, 3.38, and 5.06).

Based on personal sampling of asbestos during 1987, exposures were estimated and time-weighted annual mean exposures were classified on a scale of three degrees (0, 1, and 2). Cumulative exposures for both diesel exhaust and asbestos were calculated by multiplying the level of exposure by the duration of every work period. An exposure index was calculated by

adding for every individual contributions from all work periods for both diesel exhaust and asbestos. Four diesel exhaust index classes were created: 0 to 10, 10 to 20, 20 to 30, and >30. The four asbestos index classes were 0 to 20, 20 to 40, 40 to 60, and >60. The cumulative exposure indices were used for the nested case-control study.

Excesses were observed for all cancers and some other site-specific cancers using both comparison populations for the cohort mortality study, but none of them was statistically significant. Based on 17 cases, standardized mortality ratios (SMRs) for lung cancer were 122 and 115 using Stockholm occupationally active and general population, respectively. No dose-response was observed with increasing cumulative exposure. The cancer incidence study reportedly confirmed the mortality results (results not given).

The nested case-control study showed increasing risk of lung cancer with increasing exposure. Weighted linear regression gave RRs of 1.34 (95% CI = 1.09 to 1.64), 1.81 (95% CI = 1.20 to 2.71), and 2.43 (95% CI = 1.32 to 4.47) for the diesel exhaust indices 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as the comparison group. The study was based on 17 cases and six controls for each case matched on age ± 2 years. The results from conditional logistic regression were similar to those obtained by weighted linear regression, but none was statistically significant. Adjustment for asbestos exposure did not change the lung cancer risk for diesel exhaust.

The main strength of this study is the detailed exposure matrices constructed for both diesel exhaust and asbestos exposure, although they were based primarily on job tasks and very few actual measurements. There are a few methodological limitations to this study. The cohort is small and there were only 17 lung cancer deaths, thus the power is low. Exposure or outcome may be misclassified, although any resulting bias in the relative risk estimates is likely to be toward unity, because exposure classification was done independently of the outcome. Although the analysis by dose indices was done, no latency analysis was performed. Finally, data on smoking were missing, thus potentially confounding the lung cancer results. The authors suggest that even the heaviest smoking among individuals who were heavily exposed to diesel exhaust will be unable to explain the excess relative risk of 2.4 observed in this group. This may be an overstatement, however, as cigarette smoking is a very strong risk factor for lung cancer. Overall, this study provides some support to the excess lung cancer results found earlier among populations exposed to diesel exhaust.

8.2.9. Hansen (1993): A Follow-Up Study on the Mortality of Truck Drivers

This is a retrospective cohort mortality study of unskilled male laborers, ages 15 to 74 years, in Denmark, identified from a nationwide census file of November 9, 1970. The exposed

group included all truck drivers employed in the road delivery or long-haul business (14,225). The unexposed group included all laborers in certain selected occupational groups considered to be unexposed to fossil fuel combustion products and to resemble truck drivers in terms of work-related physical demands and various personal background characteristics (43,024).

Through automatic record linkage between the 1970 census register (the Central Population Register 1970 to 1980) and the Death Certificate Register (1970 to 1980), the population was followed for cause-specific mortality or emigration up to November 9, 1980. Expected number of deaths among truck drivers was calculated by using the 5-year age group and 5-year time period death rates of the unexposed group and applying them to the person-years accumulated by truck drivers. International Classification of Diseases Revision 8 was used to code the underlying cause of death. Test-based confidence intervals (CI) were calculated using Miettinen's method. A Poisson distribution was assumed for the smaller numbers, and CI was calculated based on exact Poisson distribution (Ciba-Geigy). Total person-years accrued by truck drivers were 138,302, whereas for the unexposed population, they were 407,780. There were 627 deaths among truck drivers and 3,811 deaths in the unexposed group. Statistically significant (SS) excesses were observed for all cancer mortality (SMR = 121, 95% CI = 104 to 140); cancer of respiratory organs (SMR = 160, 95% CI = 128 to 198), which mainly was due to cancer of bronchus and lung (SMR = 160, 95% CI = 126 to 200); and multiple myeloma (SMR = 439, 95% CI = 142 to 1,024). When lung cancer mortality was further explored by age groups, excesses were observed in most of the age groups (30 to 39, 45 to 49, 50 to 54, 55 to 59, 60 to 64, and 65 to 74), but there were small numbers of deaths in each group when stratified by age, and the excesses were statistically significant for the 55 to 59 (SMR = 229, 95% CI = 138 to 358) and 60 to 64 (SMR = 227, 95% CI = 142 to 344) age groups only.

As acknowledged by the author, the study has quite a few methodologic limitations. The exposure to diesel exhaust is assumed in truck drivers based on diesel-powered trucks, but no validation of qualitative or quantitative exposure is attempted. It is also not known whether any of these truck drivers or any other laborers had changed jobs after the census of November 9, 1970, thus creating potential misclassification bias in exposure to diesel exhaust. The lack of smoking data and a 36% rural population (usually consuming less tobacco) in the unexposed group further confound the lung cancer results. The follow-up period is relatively short, and a latency analysis was not attempted. At best, the findings of this study are consistent with the findings of other truck driver studies.

Table 8-1 summarizes the foregoing cohort studies.

Table 8-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Waller (1981)	Approximately 20,000 male London transportation workers Aged 45 to 64 years 25 years follow-up (1950-1974)	Five job categories used to define exposure Environmental benzo[a]pyrene concentrations measured in 1957 and 1979	SMR = 79 for lung cancer for the total cohort SMRs for all five job categories were less than 100 for lung cancer	Exposure measurement of benzo[a]pyrene showed very little difference between inside and outside the garage Incomplete information on cohort members No adjustment for confounding such as other exposures, cigarette smoking, etc. No latency analysis
Howe et al. (1983)	43,826 male pensioners of the Canadian National Railway Company Mortality between 1965 and 1977 among these pensioners was compared with mortality of general Canadian population.	Exposure groups classified by a group of experts based on occupation at the time of retirement Three exposure groups: Nonexposed Possibly exposed Probably exposed	RR = 1.2 (p=0.013) and RR = 1.3 (p=0.001) for lung cancer for possible and probable exposure, respectively A highly significant dose-response relationship demonstrated by trend test (p<0.001)	Incomplete exposure assessment due to lack of lifetime occupational history Mixed exposures to coal dust and diesel exhaust No validation of method was used to categorize exposure No data on smoking No latency analysis
Rushton et al. (1983)	8,490 male London transport maintenance workers Mortality of workers employed for 1 continuous year between January 1, 1967, and December 31, 1975, was compared with mortality of general population of England and Wales	100 different job titles were grouped in 20 broad categories The categories were not ranked for diesel exhaust exposure	SMR = 133 (p<0.03) for lung cancer in the general hand job group Several other job categories showed SS increased SMRs for several other sites based on fewer than five cases	Ill-defined diesel exhaust exposure without any ranking Average 6-year follow-up (i.e., not enough time for lung cancer latency) No adjustment for confounders such as smoking

Table 8-1. Epidemiological studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Wong et al. (1985)	34,156 male heavy construction equipment operators Members of the local union for at least 1 year between January 1, 1964, and December 1, 1978	20 functional job titles grouped into three job categories for potential exposure Exposure groups (high, low, and unknown) based on job description and proximity to source of diesel exhaust emissions	SMR = 166 (p<0.05) for liver cancer for total cohort SMR = 343 (observed = 5, p<0.05) for lung cancer for high-exposure bulldozer operators with 15-19 years of membership, 20+ years of follow-up SMR = 119 (observed = 141, p<0.01) for workers with no work histories	No validation of exposure categories, which were based on surrogate information Incomplete employment records Employment history other than from the union not available No data on confounders such as other exposures, smoking, etc.
Edling et al. (1987).	694 male bus garage employees Follow-up from 1951 through 1983 Mortality of these men was compared with mortality of general population of Sweden	Three exposure groups based on job titles: High exposure, bus garage workers Intermediate exposure, bus drivers Low exposure, clerks	No SS differences were observed between observed and expected for any cancers by different exposure groups	Small sample size No validation of exposure No data on confounders such as other exposures, smoking, etc.
Boffetta and Stellman (1988)	46,981 male volunteers enrolled in the American Cancer Society's Prospective Mortality Study of Cancer in 1982 Aged 40 to 79 years at enrollment First 2-year follow-up	Self-reported occupations were coded into 70 job categories Employment in high diesel exhaust exposure jobs were compared with nonexposed jobs	Total mortality (SS) elevated for railroad workers, heavy equipment operators, miners, and truck drivers Lung cancer mortality (SS) elevated for miners and heavy equipment operators Lung cancer mortality (SNS) elevated among railroad workers and truck drivers Truck drivers also showed a small dose response	Exposure information based on self-reported occupation for which no validation was done Volunteer population, probably healthy population

Table 8-1. Epidemiological studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Garshick et al. (1988)	55,407 white male railroad workers Aged 40 to 64 years in 1959 Started work 10-20 years earlier than 1959	Industrial hygiene data correlated with job titles to dichotomize the jobs as "exposed" or "not exposed"	RR = 1.45 (40-44 year age group) RR = 1.33 (45-49 year age group) Both SS After exclusion of workers exposed to asbestos RR = 1.57 (40-44 year age group) RR = 1.34 (45-49 year age group) Both SS Dose response indicated by increasing lung cancer risk with increasing cumulative exposure	Years of exposure used as surrogate for dose Not possible to separate the effect of time since first exposure and duration of exposure
Gustavsson et al. (1990)	695 male workers from 5 bus garages in Stockholm, Sweden, who had worked for 6 months between 1945 and 1970 34 years follow-up (1952-1986) Nested case-control study 17 cases, six controls for each case matched on age ± 2 years	Four diesel exhaust indices were created: 0 to 10, 10 to 20, 20-30, and >30 based on job tasks and duration of work	SMRs of 122 and 115 (OA and GP), respectively, SNS Case-control study results RR = 1.34 (10 to 20) RR = 1.81 (20 to 30) RR = 2.43 (>30) All SS with 0-10 as comparison group	Exposure matrix based on job tasks (not on actual measurements) Small cohort, hence low power Lack of smoking data
Hansen (1993)	Cohort of 57,249 unskilled laborers, ages 15 to 74, in Denmark (nationwide census file) November 9, 1970 Follow-up through November 9, 1980	Diesel exhaust exposure assumed based on diesel-powered trucks	SS SMR = 160 for bronchus and lung for total population	No actual exposure data available Lack of smoking data Job changes may have occurred from laborer to driver Short follow-up period

Abbreviations: RR = relative risk; SMR = standardized mortality ratio; SNS = statistically nonsignificant; SS = statistically significant; OA = occupationally active; GP = general population.

8.3. CASE-CONTROL STUDIES OF LUNG CANCER

8.3.1. Williams et al. (1977): Associations of Cancer Site and Type 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. Of 13,179 cancer patients, a 10% random sample of all incident invasive cancers in eight areas, a total of 7,518 were successfully interviewed in the 3 years surveyed by the TNCS. These comprised 57% 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 were coded separately for main lifetime employment, recent employment, and other jobs held according to the 1970 Census Coding Scheme. 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 3,539 interviewed males and 3,937 interviewed females, 95% and 84%, respectively, listed some main employment. 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; this was done using a series of Mantel-Haenszel stratified contingency table analyses to yield odds ratios and chi-square values. Odds ratios (ORs) were computed separately for males and females, controlling for age, race, education, tobacco, alcohol, and geographic location.

A total of 432 and 128 lung cancers were present in males and females, respectively. For males, an excess risk of 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. All of these odds ratios were adjusted for age, race, education, tobacco, alcohol, and geographic location. 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, the odds ratio for lung cancer in males was 1.40 for railroad workers and 1.34 for truck drivers. Both of these excesses were statistically nonsignificant.

The strengths of the TNCS interview data set are its large size, histological confirmation of nearly 95% of diagnoses, availability of information on occupation, and details of confounding variables obtained by personal interview and ability to control for them. Among its weaknesses are a 47% nonresponse rate and the fact that the population surveyed came from predominantly urban areas and 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. The control group was the combined "other cancers," which may have diluted the association because diesel exhaust is also suspected of being associated with bladder cancer, and this category was included in the control group when the comparison was made with lung cancer. The study presented several tables, but the total population in each table was different and never added up to the initial number interviewed. The authors failed to explain these omissions. Further, when multiple comparisons are made, some significant associations arise by chance. This analysis suggests an association with lung cancer for three industries with potential diesel exhaust exposure: trucking, railroading, and mining.

8.3.2. 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 years old were ascertained from 18 participating hospitals in six U.S. cities, 12 months prior to the interview. Eligible controls, patients at the same hospitals without tobacco-related diseases, were matched to cases by age (±5 years), race, hospital, and hospital room status. The number of male lung cancer cases interviewed totaled 502, which was 64% of those who met the study criteria for eligibility. Of the remaining 36%, 8% refused, 21% were too ill or had died, and 7% were unreliable. Seventy-five percent of eligible controls completed interviews. Of these interviewed controls, 49.9% were from the all-cancers category, whereas 50.1% 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% 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 the National Institute for Occupational Safety and Health (NIOSH) criteria to analyze occupations by diesel exposure. 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% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10 to 19% of their employees exposed were defined as "moderate exposure," and those with less than 10% of their employees exposed were defined as "low exposure."

Cases and controls were compared with respect to exposure. The relative risk was 2.0 (95% 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. Analysis by NIOSH criteria found a nonsignificant relative risk of 1.7 in the high-exposure group. There were no significantly increased cancer risks by occupation either by the first method or by the NIOSH method. To assess any possible synergism between diesel exhaust exposure and smoking, the lung cancer risks were calculated for different smoking categories. The relative risks were 1.46 among nonsmokers and ex-smokers, 0.82 among current smokers of ≤20 cigarettes/day, and 1.3 among current smokers of 20+ cigarettes/day, indicating a lack of synergistic effects.

The major strength of this study is the availability of a detailed smoking history for all the study subjects. However, this is offset by the lack of diesel exhaust exposure measurements, use of a poor surrogate for exposure, and lack of consideration of latency period. 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. Further, occupational histories were obtained from self-reports and were not validated with work records. This could have resulted in recall bias and misclassification of exposure status.

8.3.3. 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 northernmost counties of Sweden, with a total male population of about 390,000. The rural municipalities with 15% to 20% 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-year 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%), 582 surrogates of dead controls (96%), and 453 living controls (97%).

Occupational data were collected on occupations or employment held for at least 1 year 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 ratios 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 year in a specific occupation was in the analysis assigned to that occupation.

Using dead controls, the odds ratios adjusted for smoking were 1.0 (95% CI = 0.7, 1.5) and 2.7 (95% CI = 1.0, 8.1) for professional drivers (≥1 year of employment) and underground miners (≥1 year of employment), respectively. For 20 or more years of employment in those occupations, the odds ratios adjusted for smoking were 1.2 (95% CI = 0.6, 2.2) and 9.8 (95% CI = 1.5, 414). 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% CI = 1.6, 3.6) for ≥1 year of employment and 3.6 (95% CI = 1.9, 7.2) for ≥20 years of employment. All the odds ratios were calculated by adjusting for age, smoking, and municipality. After comparison with the live controls, the odds ratios were found to be lower than those observed with dead controls. None of the odds ratios were statistically significant in this comparison.

This study did not detect any excess risk of lung cancer for professional drivers, who, among all the occupations listed, had the most potential for exposure to motor vehicle exhaust. However, it is not known whether 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 was due to diesel emissions from engines or from radon daughters in poorly ventilated mines. Although a high response rate (98%) was obtained by the postal questionnaires, the use of surrogate respondents is known to lead to misclassification errors that can bias the odds ratio to 1.

8.3.4. Lerchen et al. (1987): Lung Cancer and Occupation in New Mexico

This is a population-based case-control study conducted in New Mexico that examines the association between occupation and occurrence of lung cancer in Hispanic and non-Hispanic whites. Cases involved residents of New Mexico, 25 through 84 years of age and 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-year 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% for cases. Next of kin provided interviews for 50% and 43% of male and female cases, respectively. Among controls, only 2% of the interviews were provided by next of kin 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 months since 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, an 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 year 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 analyzed 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% CI = 0.2, 1.6). Elevated odds ratios were found for uranium miners (OR = 2.8, 95% CI = 1.0, 7.7), underground miners (OR = 2.4, 95% CI = 1.2, 4.4), construction painters (OR = 2.4, 95% CI = 0.6, 9.6), and welders (OR = 4.3, 95% CI = 1.6, 11.0). No excess risks were detected for the following industries: shipbuilding, petroleum refining, construction, printing, blast furnace, and steel mills; or 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. Therefore, the analyses were presented for males only.

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 did 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.

8.3.5. Garshick et al. (1987): A Case-Control Study of Lung Cançer 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 was approximately 650,000 active and retired male U.S. railroad workers with 10 years or more of railroad service who were born in 1900 or later. 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 10 years or more of service. Information on deaths that occurred between March 1, 1981, and February 28, 1982, was obtained from the RRB. For 75% of the deceased population, death certificates were obtained from the RRB, and, for the remaining 25%, they were obtained from the appropriate state departments of health. Cause of death was coded according to the eighth revision of the ICD. 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 birth were within 2.5 years of the date of birth of the case and whose dates of death were within 31 days of the date of death noted in the case. Controls were then 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 the 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% of the locomotives in the United States were diesel powered. Investigators acknowledge that because the transition to diesel-powered engines took place in the early 1950s, some workers 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% of the 15,059 deaths reported by the RRB, from which 1,374 cases of lung cancer were identified. Fifty-five cases of lung cancer were excluded from the study for either incomplete data (20) or refusal by two States to use information on death certificates to contact the next of kin. Successful matching to at least one control with work histories was achieved for 335 (96%) cases ≤64 years of age at death and 921 (95%) cases ≥65 years of age at death. In both age groups, 90% of the cases were matched with two controls. There were 2,385 controls in the study, 98% were matched within ±31 days of the date of death, whereas the remaining 2% were matched within 100 days. Deaths from diseases of the circulatory system predominated among controls. Among the younger workers, approximately 60% had exposure to diesel exhaust, whereas among older workers, only 47% were exposed to diesel exhaust.

Analysis by a regression model, in which years of diesel exhaust exposure were the sum total of the number of years in diesel-exposed jobs, used as a continuous exposure variable, yielded an odds ratio of lung cancer of 1.39 (95% CI = 1.05, 1.83) for over 20 years of diesel exhaust exposure in the ≤64 years of age group. After adjustment for asbestos exposure and lifetime smoking (pack-years), the odds ratio was 1.41 (95% CI = 1.06, 1.88). Both crude odds ratio and asbestos exposure as well as lifetime smoking adjusted odds ratio for the ≥65 years of age group were not significant. Increasing years of diesel exhaust exposure, categorized as ≥20 diesel years and 5 to 19 diesel years, with 0 to 4 years as the referent group, showed significantly increased risk in the ≤64 years of age group after adjusting for asbestos exposure and pack-year category of smoking. For individuals who had ≥20 years of diesel exhaust exposure, the odds ratio was 1.64 (95% CI = 1.18, 2.29), whereas among individuals who had 5 to 19 years of diesel exhaust exposure, the odds ratio was 1.02 (95% CI = 0.72, 1.45). In the ≥65 years of age group, only 3% of the workers were exposed to diesel exhaust for more than 20 years. Relative odds for

5 to 19 years and ≥20 years 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 differences in results were seen. The interactions 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 years before death and gave an odds ratio of 1.43 (95% CI = 1.06, 1.94) adjusted for cigarette smoking and asbestos exposure, for workers with 15 years of cumulative exposure. For workers with 5 to 14 years of cumulative exposure, the relative odds were not significant.

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 good ascertainment (87%) 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, which may have exaggerated the contribution of smoking to lung cancer risk, and use of the Interstate Commerce Commission (ICC) job classification as a surrogate for exposure, which may have led 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 range associated with a job title that 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% of the cases. Last, as in all previous studies, there is a 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.

8.3.6. Benhamou et al. (1988): Occupational Risk Factors of Lung Cancer in a French Case-Control Study

This is a case-control study of 1,625 histologically confirmed cases of lung cancer and 3,091 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 years), 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 lifetime 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 listed 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 years, or >20 years), daily consumption of cigarettes (nonsmokers, <20 cigarettes a day, and ≥20 cigarettes a day), and duration of cigarette smoking (nonsmokers, <35 years, and ≥35 years). The data on occupations were coded by a panel of experts according to their own chemical or physical exposure determinations. Occupations were recorded blindly using the International Standard Classification of Occupations. Data on 1,260 cases and 2,084 controls were available for analysis. The remaining 365 cases and 1,007 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 (RR) 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% CI = 1.04, 1.47). On further analysis of this group, for occupations with potential diesel emission exposure, significant excess risks were found for motor vehicle drivers (RR = 1.42, 95% CI = 1.07, 1.89) and transport equipment operators (RR = 1.35, 95% 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 miners and quarrymen (RR = 2.14, 95% 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 or environmental exposures) and lung cancer. Although an attempt was made to

obtain complete occupational histories, the authors did not clarify whether, 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.

8.3.7. Hayes et al. (1989): Lung Cancer in Motor Exhaust-Related 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 (MER) 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 to 1979), New Jersey (1980 to 1981), and Louisiana (1979 to 1983). 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% among workers in MER 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 those with lung diseases and tobacco-related cancers.

A total of 2,291 cases of male lung cancers and 2,570 controls were eligible, and the data on occupations were collected by next-of-kin interviews for all jobs held for 6 months or more, including the industry, occupation, and number of years employed. The proportion of next-of-kin interviews varied by site between 50% in Louisiana to 85% in Florida. The coding schemes were reviewed to identify MER occupations, which included truck drivers and 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 years or more for all MER 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% CI = 1.1, 2.0), allowing for multiple MER employment, and 2.0 (95% 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 years, were 1.3 (95% CI = 1.0, 1.7) and 1.3 (95% CI = 0.9, 1.8) including and excluding multiple MER employment, respectively. 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 years had an odds ratio of 1.5 (95% 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 10 years. The only other job category that showed a statistically significant excess for lung cancer was the one that included taxi drivers and chauffeurs who worked multiple MER jobs for less than 10 years (OR = 2.5, 95% CI = 1.4, 4.8). For the same category, the risk for individuals working in that job for more than 10 years was 1.2 (95% CI = 0.5, 2.6). A statistical significant positive trend ($p<0.05$) with increasing employment of <2 years, 2 to 9 years, 10 to 19 years, and 20+ years was observed for truck drivers but not for other MER occupations. A statistically nonsignificant excess risk was also observed for heavy equipment operators, bus drivers, taxi drivers and chauffeurs, and mechanics employed for 10 years or more. All of the above-mentioned odds ratios were derived adjusted for birth cohort, usual daily cigarette use, and State of residence. 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 MER jobs are at an excess risk of lung cancer that is not explained by their smoking habits or exposures to other lung cancers. Because no information on type of engine had been collected, it was not possible to determine if the excess risk was due to exposure to diesel exhaust or gasoline exhaust or the mixture of the two. Among the study's 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 that used different occupational classification schemes.

8.3.8. Steenland et al. (1990): A Case-Control Study of Lung Cancer and Truck Driving in the Teamsters Union

Steenland et al. conducted a case-control study of lung cancer deaths in the Teamsters Union to determine the risk of lung cancer among different occupations. Death certificates were obtained from the Teamsters Union files in the central States for 10,485 (98%) male decedents who had filed claims for pension benefits and who had died in 1982 and 1983. Individuals were required to have 20 years tenure in the union to be eligible to claim benefits. Cases comprised all deaths (n = 1,288) from lung cancer, coded as ICD 162 or 163 for underlying or contributory cause on the death certificate. The 1,452 controls comprised every sixth death from the entire file, excluding deaths from lung cancer, bladder cancer, and motor vehicle accidents. Detailed information on work history and potential confounders such as smoking, diet, and asbestos exposure was obtained by questionnaire. Seventy-six percent of the interviews were provided by spouses and the remainder by some other next of kin. The response rate was 82% for cases and 80% for controls. Using these interview data and the 1980 census occupation and industry codes, subjects were classified either as nonexposed or as having held other jobs with potential diesel exhaust exposure. Data on job categories were missing for 12% of the study subjects. A second work history file was also created based on the Teamsters Union pension application that lists occupation, employer, and dates of employment. A three-digit U.S. census code for occupation and industry was assigned to each job for each individual. This Teamsters Union work history file did not have information on whether men drove diesel or gasoline trucks, and the four principal occupations were long-haul drivers, short-haul or city drivers, truck mechanics, and dock workers. Subjects were assigned the job category in which they had worked the longest.

The case-control analysis was done using unconditional logistic regression. Separate analyses were conducted for work histories from the Teamsters Union pension file and from next-of-kin interviews. Covariate data were obtained from next-of-kin interviews. Analyses were also performed for two time periods: employment after 1959 and employment after 1964. These two cut-off years reflect years of presumed dieselization; 1960 for most trucking companies and 1965 for independent driver and nontrucking firms. Data for analysis could be obtained for 994 cases and 1,085 controls using Teamsters Union work history and for 872 cases and 957 controls using next-of-kin work history. When exposure was considered as a dichotomous variable, for both Teamsters Union and next-of-kin work history, no single job category had an elevated risk. From the next-of-kin data, diesel truck drivers had an odds ratio of 1.42 (95% CI = 0.74, 2.47) and diesel truck mechanics had an odds ratio of 1.35 (95% CI = 0.74, 2.47). Odds ratios by duration of employment as a categorical variable were then estimated. For

the Teamsters Union work history data and when only employment after 1959 was considered, both long-haul ($p<0.04$) and short-haul drivers (not significant) showed an increase in risk with increased years of exposure. The length of employment categories for which the trends were analyzed were 1 to 11 years, 12 to 17 years, and 18 years or more. Using 1964 as the cutoff date, long-haul drivers continued to show a significant positive trend ($p=0.04$), with an odds ratio of 1.64 (95% CI = 1.05, 2.57) for those who worked for 13+ years, the highest category. Short-haul drivers, however, did not show a positive trend when 1964 was used as the cutoff date. Similar trend analysis was done for most next-of-kin data. A marginal increase in risk with increasing duration of employment as a truck driver ($p=0.12$) was observed. For truck drivers who primarily drove diesel trucks for 35 years or longer, the odds ratio for lung cancer was 1.89 (95% CI = 1.04, 3.42). The odds ratio was 1.34 (95% CI = 0.81, 2.22) for gasoline truck drivers and 1.09 (95% CI = 0.44, 2.66) for truck mechanics. No significant interactions between age and diesel exhaust exposure or smoking and diesel exhaust exposure were observed. All the odds ratios were adjusted for age, smoking, and asbestos in addition to various exposure categories.

The authors acknowledge several limitations of this study, which include possible misclassifications of exposure and smoking habits, as information was provided by next of kin; lack of sufficient latency to observe lung cancer excess; and a small nonexposed group (n = 120). Also, concordance between Teamsters Union and next-of-kin job categories could not be easily evaluated because job categories were defined differently in each data set. No data were available on levels of diesel exposure for the different job categories. Given these limitations, the positive findings of this study are probably underestimated.

8.3.9. Boffetta et al. (1990): Case-Control Study on Occupational Exposure to Diesel Exhaust and Lung Cancer Risk

This is an ongoing (since 1969) case-control study of tobacco-related diseases in 18 hospitals (six U.S. cities). Cases comprise 2,584 males with histologically confirmed primary lung cancers. Sixty-nine cases were matched to one control, whereas 2,515 were matched to two controls. Controls were individuals who were diagnosed with non-tobacco-related diseases. The matching was done for sex, age (±2 years), hospital, and year of interview. The interviews were conducted at the hospitals at the time of diagnosis. In 1985, the occupational section of the questionnaire was modified to include the usual occupation and up to five other jobs as well as duration (in years) worked in those jobs. After 1985, information was also obtained on exposure to 45 groups of chemicals, including diesel exhaust at the workplace or during hobby activities. A priori aggregation of occupations was categorized into low probability of diesel exhaust exposure (reference group), possible exposure (19 occupations), and probable exposure (13

occupations). Analysis was conducted based on "usual occupation" on all study subjects, and any occupation with sufficient cases was eligible for further analysis. In addition, cases enrolled after 1985 for which there were self-reported diesel exhaust exposure and detailed work histories were also analyzed separately.

Both matched and unmatched analyses were done by calculating the adjusted (for smoking and education) relative odds using the Mantel-Haenzael method and calculating the test-based 95% confidence interval using the Miettinen method. Unconditional logistic regression was used to adjust for potential confounders (the PROC LOGIST of SAS). Linear trends for risk were also tested according to Mantel.

Adjusted relative odds for possible and probable exposure groups as well as the truck drivers were slightly below unity, none being statistically significant for the entire study population. Although slight excesses were observed for the self-reported diesel exhaust exposure group and the subset of post-1985 enrollees for highest duration of exposure (for self-reported exposure, occupations with probable exposure and for truck drivers), none was statistically significant. Trend tests for the risk of lung cancer among self-reported diesel exhaust exposure, probable exposure, and truck drivers with increasing exposure (duration of exposure used as surrogate for increasing dose) were nonsignificant too. Statistically significant lung cancer excesses were observed for cigarette smoking only.

The major strength of this study is availability of detailed smoking history. Even though detailed information was obtained for the usual and five other occupations (1985), no effort was made to estimate or verify the actual exposure to diesel exhaust; instead, duration of employment was used as a surrogate for dose. The numbers of cases and controls were large; however, the number of individuals exposed to diesel exhaust was relatively few, thus reducing the power of the study. This study did not attempt latency analysis either. Given these limitations, the findings of this study are unable to provide either positive or negative evidence for a causal association between diesel exhaust and occurrence of lung cancer.

8.3.10. Emmelin et al. (1993): Diesel Exhaust Exposure and Smoking: A Case-Referent Study of Lung Cancer Among Swedish Dock Workers

This is a case-control study of lung cancer drawn from the cohort defined as all-male workers who had been employed as dock workers for at least 6 months between 1950 and 1974. In the population of 6,573 from 20 ports, there were 90 lung cancer deaths (cases), identified through Swedish death and cancer registers, during the period of 1960 to 1982. Of these 90 deaths, the 54 who were workers at the 15 ports for which exposure surrogate information was available were chosen for the case-control study. Four controls, matched on port and age, were

chosen for each case from the remaining cohort who had survived to the time of diagnosis of the case. Both live and deceased controls were included. The final analyses were done on 50 cases and 154 controls who had complete information on employment dates and smoking data. The smoking strata were created by classifying ex-smokers as nonsmokers if they had not smoked for at least 5 years prior to the date of diagnosis of the case; otherwise they were classified as smokers.

Relative odds and regression coefficients were calculated using conditional logistic regression models. Comparisons were made both with and without smoking included as a variable, and the possible interaction between smoking and diesel exhaust was tested. Both weighted linear regressions of the adjusted relative odds, and the regression coefficients were used to test mortality trends with all three exposure variables.

Exposure to diesel exhaust was assessed indirectly by initially measuring (1) exposure intensity based on exhaust emission, (2) characteristics of the environment in terms of ventilation, and (3) measures of proportion of time in higher exposed jobs. For exhaust emissions, annual diesel fuel consumption at a port was used as the surrogate. For ventilation, the annual proportion of ships with closed or semiclosed holds was used as the surrogate. The proportion of time spent below decks was used as the surrogate for more exposed jobs. Although data were collected for all three measures, only the annual fuel consumption was used for analysis. Because every man was likely to rotate through the various jobs, the authors thought using annual consumption of diesel fuel was the appropriate measure of exposure. Consequently, in a second analysis, the annual fuel consumption was divided by the number of employees in the same port that year to come up with the fuel-per-person measure, which was further used to create a second measure, "exposed time." The "annual fuel" and exposed-time data were entered in a calendar time-exposure matrix for each port, from which individual exposure measures were created. A third measure, "machine time" (years of employment from first exposure), was also used to compare the results with other studies. All exposure measures were accumulated from the first year of employment or first year of diesel machine use, whichever came later. The last year of exposure was fixed at 1979. All exposures within 2 years prior to the date of lung cancer diagnosis were omitted from both cases and matched controls. A priori classification into three categories of low, medium, and high exposure was done for all three exposure variables, machine time, fuel, and exposed time.

Conditional logistic regression models, adjusting for smoking status and using low exposures and/or nonsmokers as a comparison group, yielded positive trends for all exposure measures, but no trend test results were reported, and only the relative odds for the exposed-time exposure measure in the high-exposure group (OR = 6.8, 90% CI = 1.3 to 34.9) was reported as

statistically significant. For smokers, adjusting for diesel exhaust exposure level, the relative odds were statistically significant and about equal for all the three exposure variables—machine time, OR = 5.7 (90% CI = 2.4 to 13.3); fuel, OR = 5.5 (90% CI = 2.4 to 12.7); and exposed time, OR = 6.2 (90% CI = 2.6 to 14.6). Interaction between diesel exhaust and smoking was tested by conditional logistic regression in the exposed-time variable. Although there were positive trends for both smokers and nonsmokers, the trend for smokers was much steeper—low, OR = 3.7 (90% CI = 0.9 to 14.6); medium, OR = 10.7 (90% CI = 1.5 to 78.4); and high, OR = 28.9 (90% CI = 3.5 to 240)—indicating more than additive interaction between these two variables.

In the weighted linear regression model with the exposed-time variable, the results were similar to those using the logistic regression model. The authors also explored the smoking variable further in various analyses, some of which suggested a strong interaction between diesel exhaust and smoking. However, with just six nonsmokers and no further categorization of smoking amount or duration, these results are of limited value.

The diesel exhaust exposure matrices created using three different variables are intricate. Analyses by any of these variables essentially yield the same positive results and positive trends, providing consistent support for a real effect of diesel exhaust exposure, at least in smokers. However, there are some methodological limitations to this study that prevent a more definitive conclusion. The numbers of cases and controls are small. There are very few nonsmokers, thus testing the effects of diesel exhaust exposure in them is futile. Lack of information on asbestos exposure, to which dock workers are usually exposed, may also confound the results. Also, no latency analyses are presented. Overall, despite these limitations, this study supports the earlier findings of excess lung cancer mortality among individuals exposed to diesel exhaust.

Table 8-2 summarizes the above lung cancer case-control studies.

8.4. CASE-CONTROL STUDIES OF BLADDER CANCER

8.4.1. 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% of them were interviewed at home. A total of 480 male and 152 female case-control pairs were available for analysis. For each case, one neighborhood control, matched by age (±5 years) and sex, was also interviewed at home to obtain data on smoking, occupation, dietary sources of nitrites and nitrates that convert to

Table 8-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Williams et al. (1977)	7,518 (3,539 males and 3,979 females) incident invasive cancers from the Third National Cancer Survey Lung cancer cases: 32 in males 28 in females Combined other cancer sites were used as controls	Main lifetime, recent, and other employment information obtained at the time of survey 1970 Census Coding Scheme for Employment was used to code the occupations by one of the authors	SNS elevated relative odds were observed among occupations of trucking, railroading, and mining	Exposure estimation based on self-report that was not validated 47% nonresponse Control group consisted of other cancers, probably diluting the risk estimation Small numbers in cause-specific cancers and individual occupations
Hall and Wynder (1984)	502 histologically confirmed lung cancers Cases diagnosed 12 mo prior to interviews 502 matched hospital controls without tobacco-related diseases, matched for age, sex, race, and geographical area Population from 18 hospitals in controls	Based on previous Industrial Hygiene Standards for a particular occupation, usual lifetime occupation coded as "probably high exposure" and "no exposure" NIOSH standards used to classify exposures: High Moderate Low	SNS excess risk after adjustment for smoking for lung cancer: RR = 1.4 (1st criteria) and RR = 1.7 (NIOSH criteria)	Complete lifetime employment history not available Self-reported occupation history not validated No analysis by dose, latency, or duration of exposure No information on nonoccupational diesel exposure

Table 8-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Damber and Larsson (1987)	589 lung cancer cases who had died prior to 1979 reported to Swedish registry between 1972 and 1977 582 matched dead controls (sex, age, year of death, municipality) drawn from National Registry of Cause of Death 453 matched living controls (sex, year of birth, municipality) drawn from National Population Registry	Occupations held for at least 1 year or more Using a 5-digit code the occupations were classified according to Nordic Classification of Occupations	SS OR = 2.7 (≥1 year of employment) SS OR = 9.8 (≥20 years of employment) Adjustment for smoking was done SNS OR = 1.2 for professional drivers (≥20 years of employment) with dead controls SNS OR = 1.1 ≥20 years of employment) with living controls	Uncertain diesel exhaust exposure No validation of exposure done Underground miners data not adjusted for other confounders such as radon, etc.
Lerchen et al. (1987)	506 lung cancer cases from New Mexico tumor registry (333 males and 173 females) Aged 25-84 years Diagnosed between January 1, 1980, and December 31, 1982 771 (499 males and 272 females) frequency matched with cases, selected from telephone directory	Lifetime occupational history and self-reported exposure history were obtained Coded according to Standard Industrial Classification Scheme	No excess of relative odds was observed for diesel exhaust exposure	Exposure based on occupational history and self-report, which was not validated 50% occupational history provided by next of kin Absence of lung cancer association with asbestos suggests misclassification of exposure

Table 8-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Garshick et al. (1987)	1,319 lung cancer cases who died between March 1, 1981, and February 28, 1982 2,385 matched controls (two each, age and date of death) Both cases and controls drawn from railroad worker cohort who had worked for 10 or more years	Personal exposure assessed for 39 job categories This was corrected with job titles to dichotomize the exposure into: Exposed Not exposed	SS OR = 1.41 (≤64 year age group) SS OR = 1.64 (≤64 year age group) for ≥20 years diesel exhaust exposure group when compared to 0- to 4-year exposure group All ORs adjusted for lifetime smoking and asbestos exposure	Probable misclassification of diesel exhaust exposure jobs Years of exposure used as surrogate for dose 13% of death certificates not ascertained Overestimation of smoking history
Benhamou et al.-(1988)	1,260 histologically confirmed lung cancer cases 2,084 non-tobacco-related disease matched controls (sex, age at diagnosis, hospital admission, and interviewer) Occurring between 1976 and 1980 in France	Based on exposures determined by panel of experts The occupations were recorded blindly using International Standard Classification of Occupations as chemical or physical exposures	Significant excess risks were found in motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35) (smoking adjusted)	Exposure based on occupational histories not validated Exposures classified as chemical and physical exposure, not specific to diesel exhaust
Hayes et al. (1989)	Pooled data from three different studies consisting of 2,291 male lung cancer cases 2,570 controls	Occupational information from next of kin for all jobs held Jobs classified with respect to potential exposure to known and suspected pulmonary carcinogens	SS OR = 1.5 for truck drivers (>10 years of employment) SS positive trend with increasing employment as truck driver	Exposure data based on job description given by next of kin, which was not validated Could have been mixed exposure to both diesel and gasoline exhausts Job description could have led to misclassification

Table 8-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Steenland et al. (1990)	1,058 male lung cancer deaths between 1982 and 1983 1,160 every sixth death from entire mortality file sorted by social security number (excluding lung cancer, bladder cancer, and motor vehicle accidents) Cases and controls were from Central State Teamsters who had filed claims (requiring 20-year tenure).	Longest job held: diesel truck driver, gasoline truck driver, both types of trucks, truck mechanic, and dock workers	As 1964 cut-off point: SS OR = 1.64 for long-haul drivers with 13+ years of employment Positive trend test for long-haul drivers (p=0.04) SS OR = 1.89 for diesel truck drivers of 35+ years of employment	Exposure based on job titles not validated Possible misclassification of exposure and smoking, based on next-of-kin information Lack of sufficient latency
Boffetta et al. (1990)	From 18 hospitals (since 1969) 2,584 male lung cancer cases matched to either one control (69) or two controls (2,515) were drawn. Matched on age, hospital, and year of interview	A priori aggregation of occupations categorized into low probability, possible exposure (19 occupations), and probable exposure (13 occupations) to diesel exhaust	OR slightly below unity SNS	No verification of exposure Duration of employment used as surrogate for dose Number of individuals exposed to diesel exhaust was small
Emmelin et al. (1993)	50 male lung cancer cases from 15 ports (worked for at least 6 months between 1950 and 1974), 154 controls matched on age and port	Indirect diesel exhaust exposure assessment done based on (1) exposure intensity, (2) characteristics of ventilation, (3) measure of proportion of time in higher exposure jobs	SS OR for high-exposure group = 6.8	Numbers of cases and controls are small Very few nonsmokers Lack of exposure information on asbestos No latency analysis

Abbreviations: OR = odds ratio; RR = relative risk; SNS = statistically nonsignificant; SS = statistically significant.

nitrosamines (nonpublic 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 chronological 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 workplace. Relative risk estimates were computed using the linear logistic model applied to individually matched case-control pairs.

A baseline comparison of cases and controls showed that, whereas 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 the chemical (RR = 7.5, 95% CI = 1.7, 67.6), rubber (RR = 5.0, 95% CI = 0.6, 236.5), petroleum (RR = 5.3, 95% CI = 1.5, 28.6), medicine (RR = 2.6, 95% CI = 0.9, 9.3), and spray painting (RR = 1.8, 95% CI = 0.7, 4.6) industries. The excess risks were statistically significant only for the petroleum and chemical industries. 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. Among males, statistically nonsignificant excess risks were 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% CI = 0.8, 11.8). Statistically significant excess risks were observed for railroad workers (RR = 9.0, 95% CI = 1.2, 394.5) and welders (RR = 2.8, 95% 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% CI = 1.1, 7.6), metal recorders (RR = 2.6, 95% CI = 1.0, 7.3), and nursery men (RR = 5.5, 95% CI = 1.2, 51.1). Statistically nonsignificant excesses were also detected for exposure to two chemicals: benzidine and its salts, RR = 1.3, and *bis*-chloromethyl 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 of cigarettes, and average frequency of cigarettes per day. In males the highest significant risk was observed for latency of less than 35 years; after that time the risk reduced slightly with increasing latency. In females the highest significant risk was for more than 35 years 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 (nitrates and nitrites) 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 who reported having "ever been exposed" versus "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, the authors used the a priori high-risk occupations; hence, nothing can be concluded about exposure to diesel exhaust for occupations that were not part of that list. The authors provided no explanation on possible selection bias, as only 77% of the eligible population was included in the study.

8.4.2. 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 six U.S. cities between January 1981 and May 1983. Cases were individuals with histologically confirmed primary cancer of the bladder, diagnosed within 12 months prior to the interview. Controls were individuals with non-tobacco-related diseases who were matched to the cases by age (within 8 years), race, year of interview, and hospital of admission. Women were excluded from the study because 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 operators and repairmen, railroad workers, and warehousemen. In the second method, guidelines set by NIOSH were used to classify occupations based on exposure to diesel exhaust. 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% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10% to 19% of their employees exposed as "moderate exposure," and those with less than 10% of their employees 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% and 72% in cases and controls, respectively.

A total of 194 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 based on two cases and three controls (95% CI = 0.34, 11.61), the relative odds were less than 1 for other diesel exhaust exposure occupations such as bus and truck drivers, warehousemen, material handlers, and heavy equipment workers. 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 were controls. Current smokers of 1 to 20 cigarettes/day had relative odds of 3.64 (95% CI = 2.04, 6.49), current smokers of 21+ cigarettes/day had relative odds of 3.51 (95% CI = 2.00, 6.19), while ex-smokers had relative odds of 1.72 (95% 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 occupations without diesel exhaust exposure. A synergistic effect between the two also was not detected.

This study has two major methodologic limitations, both pertaining to exposure classification. First, the use of "usual" occupation may have led 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 of dose-response relationships 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 lifestyle factors specific to the truck-driving occupation.

8.4.3. 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 (eighth revision of the ICD) 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 years), and year of death. The second for 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) for controls. Information on demographic characteristics, lifetime occupational and residential histories, tobacco use, diet, and medical history was obtained on each subject. The odds ratio was calculated to ascertain a measure of association between truck driving and bladder cancer. Because separate analyses of the two control series gave similar results, the two control series were combined. Also, because matched analyses yielded results similar to those provided by the unmatched analyses, results of the unmatched analyses were presented.

Sixteen percent (35) of the cases and 12% (53) of the controls had been employed as truck drivers, yielding an odds ratio of 1.5 (95% CI = 0.9, 2.6) after adjustment for county of residence and age at death. For New Hampshire, the odds ratio was 1.3 (95% CI = 0.7, 2.3), and for Vermont, the odds ratio was 1.7 (95% 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 of increasing relative odds with increasing duration of employment. The trend test was statistically significant (p=0.006). The odds ratio was statistically significant for the 5 to 9 years of employment category only (OR = 2.9, 95% CI = 1.2, 6.7). Similarly, analysis by calendar year first employed showed a statistically significant

odds ratio for 1930 to 1949 (OR = 2.6, 95% CI = 1.3, 5.1), whereas relative odds were not significant if subjects 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 odds ratio of 1.8 (95% CI = 0.5, 7.0) was derived for those who were exposed to diesel exhaust during their truck-driving jobs as compared to an odds ratio of 1.5 (95% CI = 0.8, 2.7) for those not reporting diesel exhaust exposure. Analysis by duration of exposure (0, 1 to 19 years, 20 to 29 years, 30 to 39 years, and 40+ years) to diesel fuel or engines in other occupations, which were "self-reported" by participants, showed a statistically significant positive trend (p=0.024) for bladder cancer, although none of the individual odds ratios in these duration categories were statistically significant.

This study investigated an association between truck driving and bladder cancer in an attempt to understand the reasons for the high rates of bladder cancer in rural areas 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 to conclude that the 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 was 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.

8.4.4. Steenland et al. (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 provide data on occupations and employers and are compiled from a door-to-door yearly census of all residents 18 years old and older. The directories are available in most medium-size cities in the United States. A unique feature of city directory data is that they identify specific employers, and as the authors suggest, this information may be better than death certificates for rapid, inexpensive, record-based epidemiologic studies.

A case-control study was conducted of male bladder cancer deaths in Hamilton County (including Cincinnati), OH. This county was selected because it is in an industrialized area with high bladder cancer rates and also because city directories cover approximately 85% of the people living in the 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, that 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 years), 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 1,275 controls.

The death certificate analysis involved all 731 cancer deaths, with two 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 three-digit U.S. census code using the method adopted by the U.S. Bureau of the Census. Five 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 years) for truck drivers (OR = 12, p=0.01) and furnace operators (based on four 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 years of employment, OR = 2.21, p<0.05). Both truck driving and railroad industry occupations involve diesel emission exposures.

The analysis of 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% 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 certificates for 68.3% of the study subjects.

This study demonstrated that city directories are a relatively inexpensive and accessible source of occupational data for epidemiologic studies. Limitations of this study 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. Although 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. The occupational data were missing for 15%, whereas employer data were missing for 36% in the city directory. 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, because survival for bladder cancer is high, and the absence of data on confounding factors such as smoking, beverage consumption, and medication use.

8.4.5. 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 for less than 5 years were excluded. Of the 120 cases eligible to participate, 1 died prior to the interview, 2 refused to participate, and the remaining 117 cases, representing 60% 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 years). Of the 117 cases, 99 were males and 18 were females. Hospital controls, selected from the same hospital as the cases, were hospitalized for the first time within 3 months of diagnosis of the illness of the cases. 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 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, socioeconomic, 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 were 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 2 years previously. Ex-smokers were those who ceased smoking at least for 2 years or more than 2 years previously. For each subject a cumulative lifelong consumption of cigarettes by type was estimated, and an average consumption of cigarettes/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 versus those who were never employed in that occupation. Socioeconomic status of cases and neighborhood controls 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, whereas that for oil refinery workers increased after adjusting for smoking (no RRs were presented). The adjusted relative risks 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 relative risk in males for current smokers relative to nonsmokers was 7.2 (95% 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 relative risk of 12 (95% 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% of the cases registered in the cancer registry were interviewed, and no information was provided for the remaining 40% eligible nonrespondents to determine if the study sample was selectively biased in any way. The sample size of 117 was small, and the analysis of males reduced it to 99. Although 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 attempted 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 showing the distribution of cases and controls for selected activities or professions did not indicate 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. Although rare in the La Plata area, the occupations known to be associated with bladder cancer (dye, rubber, leather, and textile workers) are acknowledged by the authors.

8.4.6. Iyer et al. (1990): Diesel Exhaust Exposure and Bladder Cancer Risk

This study is a hospital-based case-control study of bladder cancer and potential exposure to diesel exhaust using data from a large ongoing case-control study of tobacco-related neoplasms conducted by the American Health Foundation. An earlier study by Wynder et al. (1985) looked at the relationship between occupational exposure to diesel exhaust and the risk of bladder cancer. For this study, the objective was to evaluate the relationship between the different measures of exposure to diesel exhaust, occupational and self-reported, and the risk of bladder cancer. Cases comprised 136 patients with histologically confirmed primary cancer of the urinary bladder interviewed at 18 hospitals in six U.S. cities. Two controls were selected per

case, matched for sex, age (within 2 years), race, hospital, and year of interview. A total of 160 controls had non-tobacco-related malignancies distributed as follows: stomach cancer (6%), colorectal cancer (20%), prostate cancer (6%), and leukemia or lymphoma (8%). Among the 112 controls with nonmalignant diseases, 3% had benign neoplasms, 6% had hyperplasia of the prostate, and 6% had dorsopathies. Distribution of the other nonmalignant illnesses was not provided. Occupational history included information on usual occupation and up to five other jobs. Exposure to diesel exhaust in hobby activities also was collected. For the purpose of this analysis, occupations were aggregated a priori into three categories: low probability of exposure (reference group), possible exposure, and probable exposure. Analyses were also done for self-reported exposure to diesel exhaust. Risk estimates were obtained by unconditional logistic regression using PROC LOGIST of SAS. Cases and controls were first compared by age, race, education, and smoking habit. Cases were found to be less educated than controls ($p<0.05$). Crude odds ratios for diesel exhaust exposure, based on occupational or self-reported exposure, were not significantly elevated after controlling for smoking and educational status (OR = 1.2, 95% CI = 0.8, 2.0). When individual occupations were analyzed separately, truck drivers showed no excess risk (OR = 0.48, 95% CI = 0.15, 1.56).

The authors concluded that their study does not support the hypothesis of an association between exposure to diesel exhaust and bladder cancer. Several significant limitations of exposure assessment and analysis are evident in this study. In the introduction, the authors stated that they refined the definition of exposure to diesel exhaust by obtaining a lifetime occupational history, but in the methods section they stated that they restricted analysis to usual occupational history and five other jobs, which was not that different from their earlier study (Wynder et al., 1985). The terms, low probability of exposure, possible exposure, and probable exposure, also were not clearly defined. Information on duration of employment or exposure was not presented, and no attempts were made to validate occupational history. No information was available on calendar years of employment in the truck-driving industry or the locomotive occupations. Because diesel trucks and locomotives were introduced in the mid-1950s and the dieselization was completed by 1960, it would be important to use 1960 as a cutoff date and to restrict analysis to subjects who worked in these industries after that date. No information on nonrespondent cases and controls was provided. The authors indicated in the methods section that cases were individually matched to controls, but they performed an unmatched analysis to calculate the odds ratios and did not address why they did not preserve the matching in the analysis, especially because such an analysis could bias the risk estimates to unity.

8.4.7. Steineck et al. (1990): Increased Risk of Urothelial Cancer in Stockholm From 1985 to 1987, After Exposure to Benzene and Exhausts

This study was conducted to investigate the association between benzene, diesel, and petrol exhausts as well as some other industry-related chemicals and the risk of urothelial cancer. Designed as a population-based case-control study, it was conducted among all men born between 1911 and 1945 and living in the County of Stockholm for all or part of the observation period (September 15, 1985, to November 30, 1987). All incident cases of urothelial cancer and squamous-cell carcinoma of the lower urinary tract were contacted for inclusion in the study. Controls were selected by stratified random sampling during the observation period from a computerized register for the population of Stockholm. A postal questionnaire was sent to study subjects at their homes to collect information on occupational history. The questions on occupation included exposure to certain specified occupations/industries/chemicals and lists of all jobs held and duration in each job. An industrial hygienist, unaware of case-control status, classified subjects as being exposed or unexposed to 38 agents and groups of substances, including 17 exposure categories with aromatic amines. Using all the exposure information, the exposure period was defined and the annual dose was rated as low, moderate, or high based on the accumulated dose (exposure duration multiplied by intensity of exposure) during the course of 1 average year for the defined exposure period. Swedish and international data were used to classify subjects as exposed, based on air concentrations in the work environment that were higher than for the general public, or skin contact with liquids of low volatility. To allow for latency, the authors ignored exposures after 1981. Data were gathered from 256 cases and 287 controls. Controls were selected by stratified random sampling four times from the computerized register during the observation period of the population of the County of Stockholm. These subjects comprised 80% of eligible cases and 79% of eligible controls. Nine of the cases and 16% of the controls refused to participate in the study. The distribution of urothelial cancers was as follows: 5 tumors in the renal pelvis, 243 in the urinary bladder, 5 in the ureter, none in the urethra, and 3 at multiple sites. Two cases who were exposed to a high annual dose of aromatic amines were omitted from all further analysis to eliminate their confounding effects. Crude relative risks were calculated for men classified as exposed or not exposed to several substances. Twenty-five cases and 19 controls reported having been exposed to diesel exhaust, yielding an odds ratio of 1.7 (95% CI = 0.9, 3.3). The corresponding relative odds for petrol exhausts, based on 24 cases and 24 controls, were 1.0 (95% CI = 0.5, 1.9). Odds ratios were then calculated for low, moderate, and high levels of the annual dose adjusted for smoking and year of birth. For diesel exhausts, the odds ratio was 1.3 (95% CI = 0.6, 3.1) for low levels, 2.2 (95% CI = 0.7, 6.6) for moderate levels, and 2.9 (95% CI = 0.3, 30.0) for high levels, indicating a dose response.

The corresponding odds ratios for petrol exhausts were 0.6 (95% CI = 0.3, 1.3), 1.4 (95% CI = 0.5, 3.7), and 3.9 (95% CI = 0.4, 35.5). Restricting the analysis to only moderate or high annual doses of exposure adjusted for year of birth and smoking showed a sevenfold increased risk for subjects exposed to both diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8). For exposure to diesel (OR = 1.1) and petrol (OR = 1.0) exhausts alone, no excess risk was detected in this analysis. Odds ratios were calculated for low, moderate, and high exposure to benzene, with rates of 1.7 (95% CI = 0.6, 5.1) for low annual doses, 1.1 (95% CI = 0.3, 4.5) for moderate annual doses, and 3.0 (95% CI = 1.0, 8.7) for high annual doses.

The authors discuss misclassification and confounding as sources of bias in this study. To examine misclassification they compared hygienist-assessed exposure and self-reported exposure for printing ink and found a higher relative risk and fewer exposed subjects for hygienist-assessed exposure, indicating that specificity was a problem for self-reported exposure. It is not known to what extent this may have affected the risk estimates for diesel exhausts since data on self-reported exposure to diesel are not presented. They also mention the possibility of exposure misclassification from using an average annual dose in which a person exposed to an agent at a high level for a few working days and a person exposed to a low level for many days are both rated as exposed to low annual doses. Although statistically nonsignificant elevated odds ratios of 1.3, 2.3, and 2.9 were derived for low, moderate, and high levels of diesel exposure, the authors state that some of their subjects may have later worked in jobs with benzene exposure, and because an elevated risk was detected for benzene exposure, this confounding effect may explain some of the excess risk. An almost statistically significant interaction was observed for exposure to combined diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8), which changed to 5.1 (95% CI = 0.6, 43.3) after adjustment for benzene exposure, again providing evidence for the confounding role of benzene exposure in explaining some of the observed results.

Table 8-3 summarizes the bladder cancer case-control studies.

8.5. DISCUSSION AND SUMMARY

Certain extracts of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic in animals and in humans. Animal data suggest that diesel exhaust is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods of time. Because large working populations are currently exposed to diesel exhaust and because nonoccupational ambient 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.

Table 8-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Howe et al. (1980)	480 male case-control pairs 152 female case-control pairs Cases diagnosed between April 1974 and June 1976 in three Canadian provinces Matched on age and sex	Based on occupational history of jobs involving exposure to dust and fumes A priori suspect industries	SNS RR = 2.8 for diesel and traffic fumes SS RR = 9.00 for railroad workers	Exposure based on occupational history, which was not validated Diesel exhaust and traffic fumes were combined Only 77% of eligible population included in the study
Wynder et al. (1985)	194 histologically confirmed male cases between the ages of 20 and 80 years 582 matched controls (age, race, year of interview, and hospital of admission); diseases not related to tobacco use From 18 hospitals located in six U.S. cities between January 1981 and May 1983	Occupational titles were defined by Industrial Hygiene Standard into dichotomous "exposed" and "not exposed" Also defined by NIOSH standards into "high exposure," "moderate exposure," and "low exposure"	SNS ORs were 1.68 and 0.16 for high and moderate exposure, respectively, as compared to low exposure	Exposure based on usual occupation may have led to misclassification Dichotomous classification made dose-response analysis unattainable No data on other confounders such as smoking

Table 8-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Hoar and Hoover (1985)	Population-based, case-control study 325 cases from the residents of New Hampshire and Vermont who died of bladder cancer between 1975 and 1979 A total of 673 controls were chosen from other deaths during the same time period Two matched controls (age, sex, race, state, year of death, and second one matched also on county of residence)	Lifetime occupational history obtained from next of kin	SS OR = 2.9 for 5 to 9 years of employment as truck driver but not for ≥10 years of employment Positive trend (p=0.006) observed with increasing duration of employment as truck driver	Exposure defined as occupation of "truck driver" (i.e., it could have been diesel or gasoline or both) No histological confirmation of bladder cancer diagnosis No data on other confounders such as other exposures, smoking, etc.
Steenland et al. (1987)	648 male bladder cancer deaths from Hamilton County, OH 1,275 matched controls from other deaths (pool of six controls for each case, excluding urinary tract tumors and pneumonias matched on sex, age at death, year of death, race)	Occupation or industry listed in city directory and on death certificates	OR = 12 (p=0.01) for truck drivers with ≥20 years of employment OR = 2.21 (p≤0.05) for railroad workers with >20 years of employment	Exposure based on city directory or death certificate listing that was not validated Lack of controlling for confounders City directory usually has short-term job listing Missing data on 15% of occupations and 36% for employers in the directory

Table 8-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Iscovich et al. (1987)	117 histologically confirmed bladder cancer cases (60% of all incident cases) 117 hospital controls and 117 neighborhood controls (matched on age and sex) Cases and hospital controls from 10 general hospitals in greater La Plata between March 1983 and December 1985	Past and present occupational data were collected by questionnaire An exposure index based on a job exposure matrix was generated	SS OR = 4.3 for truck and railway drivers SS RR = 6.2 for oil refinery workers	Exposure based on job held that was not validated 40% of eligible cases were nonrespondent Small sample size Interviewers were not "blind" to the status of an individual, and this could have biased the findings Truck and railroad drivers were grouped together Not adjusted for other confounding exposures such as dye, rubber, etc.
Iyer et al. (1990)	136 histologically confirmed bladder cancer cases 272 controls, two each matched on sex, age, race, hospital, and year of interview (160 malignant, 112 nonmalignant) From 18 hospitals in six U.S. cities	Lifetime occupational history Self-reported diesel exhaust exposure Exposure aggregated a priori into: Low probability Possible Probable	No excess found	Exposure based on self-report, which was not validated Although lifetime occupational history was obtained, analysis was restricted to usual occupation A priori classification was ambiguous

Table 8-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Steineck et al. (1990)	Population-based study from County of Stockholm Men born between 1911 and 1945 256 (243 bladder) urinary tract cancer incident cases (80% of eligibles) 287 controls (79% of eligibles) from population of Stockholm Observation period September 15, 1985, to November 30, 1987	Occupational history classified into exposed and nonexposed by industrial hygienist "blind" toward case or control status Using all exposure information, annual dose rated as "low," "moderate," and "high"	SNS OR = 1.3 for low, OR = 2.2 for moderate, and OR = 2.9 for high exposure were observed for diesel exposure SNS OR = 7.1 observed for diesel and gasoline exhaust combined exposure	Elaborate exposure history classification not used to advantage by simultaneous adjustment Misclassification in exposure may have occurred Small sample size of only 25 cases and 19 controls were exposed to diesel exhaust Confounding by other exposures not accounted for, except benzene

Abbreviations: OR = odds ratio; RR = relative risks; SNS = statistically nonsignificant; SS = statistically significant.

Because diesel emissions become diluted in the ambient air, it is difficult to study the health effects in the general population. Nonoccupational exposure to diesel exhaust is worldwide in urban areas. Thus, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are nonoccupationally exposed to diesel exhaust. Furthermore, the "exposed" group in these studies is based on job titles, which in most instances are not verified or correlated with environmental hygiene measurement. The issue of health effect measurement gets further complicated by the fact that occupational cohorts tend to be healthy and have below-average mortality, usually referred to as the "healthy worker effect." Hence, the usual standard mortality ratios observed in cohort mortality studies are underestimations of real risk.

A major difficulty with the occupational studies considered here was the measurement of the actual diesel exhaust exposure. Because all the cohort mortality studies were retrospective, the assessment of health effects from exposure to diesel exhaust was naturally indirect. In these occupational settings, no systematic quantitative records of ambient air were available. 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 have been measured to assess the occupational exposure to diesel exhaust. All studies have relied on the job categories or self-report of exposure to diesel exhaust. In the studies by Garshick et al. (1987, 1988), the diesel exhaust-exposed job categories were verified on the basis of an industrial hygiene survey done by Woskie et al. (1988a,b). It was found by the investigators that in most cases the job titles were good surrogates for diesel exhaust exposure. Also, in the railroad industry where only persons who had at least 10 years of work experience were included in the study, the workers tended not to change job categories over the years. Thus, a job known only at one point in time was a reasonable marker of past diesel exhaust exposure. Unfortunately, the exposure was only qualitatively verified. The quantitative use of this information would have been much more meaningful. 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. Currently, there are about 385 underground metal mines in the United States. Of these, 250 have been permanently operating, whereas 135 have been intermittently operating (Steenland, 1986).

Approximately 20,000 miners are employed, but not all of them are currently working in the mines. Diesel engines were introduced in the metal mines in the early to mid-1960s. Although all these mines use diesel equipment, it is difficult to estimate how many of these miners were actually exposed to diesel fumes.

Diesel engines were introduced in coal mines at an even later date, and their use is still quite limited. In 1983, approximately 1,000 diesel units were in place in underground coal mines, up from about 200 units in 1977 (Daniel, 1984). The number of units per mine varies greatly; one mine may account for more than 100 units.

Even if it were possible to estimate how many miners (metal and coal) were exposed to diesel exhaust, it would be very difficult to separate out the confounding effects of other potential pulmonary carcinogens, such as radon decay products, heavy metals (such as arsenic, chromium), etc. Furthermore, the relatively short latency period limits the usefulness of these cohorts of miners.

8.5.1. The Cohort Mortality Studies

The cohort studies mainly demonstrated an increase in 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 (just 6 years in the Rushton et al. study), lack of information on confounding variables, and lack of analysis by duration of exposure, duration of employment, or latency that preclude their use in determining the carcinogenicity of diesel exhaust. Although the Waller (1981) study had a 25-year follow-up period, the cohort was restricted to 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. Metastases from the cancers of the large intestine and rectum in the liver probably were misclassified as primary liver cancer, which led to an observed excess risk. This study did demonstrate a nonsignificant positive trend for cancer of the lung with length of membership and latency. Analysis of deceased retirees showed a significant excess of lung cancer. Individuals without work histories who started work prior to 1967, when records were not kept, may have been in the same jobs for the longest period of time. Workers without job histories included those who had the same job before and after 1967 and thus may have worked about 12 to 14

years longer; these workers exhibited significant excess risks of lung cancer and stomach cancer. If this assumption about duration of jobs is correct, then these site-specific causes can be linked to diesel exhaust exposure. One of the methodological limitations of this study is that most of these men worked outdoors; thus, this cohort might have had relatively low exposure to diesel exhaust. The authors did not present any environmental measurement data either. Because of the absence of detailed work histories for 30% of the cohort and the availability of only partial work histories for the remaining 70%, 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 cannot be used to support a causal association or to refute the same between exposure to diesel exhaust and lung cancer.

A 2-year mortality analysis by Boffetta and Stellman (1988) of the American Cancer Society's prospective study, 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% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.6, 95% 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 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 methodologic limitations, such as the lack of representiveness of the study population (composed of volunteers only, who were probably healthier than the general population), leading to an underestimation of the risk and the questionable reliability of exposure data based on self-administered questionnaires that 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 by Gustavsson et al. (1990) and Hansen (1993) among bus garage workers (Stockholm, Sweden) and truck drivers, respectively. An SMR of 122 was found among bus garage workers based on 17 cases. A nested case-control study was also conducted in this cohort. Detailed exposure matrices based on job tasks were assembled for both diesel exhaust and asbestos exposures. Statistically significant increasing lung cancer relative risks of 1.34, 1.81, and 2.43 were observed for diesel exhaust indices of 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as a comparison group. Adjustment for asbestos exposure did not change the results. The main strength of this study is the detailed exposure matrices; some of the limitations are lack of smoking histories and low power (small cohort).

Hansen (1993), on the other hand, found statistically significant SMR of 160 due to cancer of bronchus and lung. No dose response was observed, although the excesses were

observed in most of the age groups (30 to 39, 45 to 49, 50 to 54, 55 to 59, 60 to 64, and 65 to 74). There are quite a few methodologic limitations to this study. Exposure to diesel exhaust was assumed in truck drivers for diesel-powered trucks, but no validation of exposure was attempted. Smoking data were lacking, follow-up period was short, and no latency analysis was done. The findings of both these studies are consistent with the findings of other truck driver studies.

Two mortality studies of railroad workers were conducted, 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 because the data are lacking, it is possible that the 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 of coal miners, but lack of data on confounders such as asbestos and smoking makes interpretation of this study difficult. When three diesel exhaust exposure categories were examined for smoking-related diseases such as emphysema, laryngeal cancer, esophageal cancer, and buccal cancer, positive trends were observed, raising a possibility that the dose-response demonstrated for diesel exposure may have been due to smoking. The findings of this study are at best suggestive of diesel exhaust being a lung carcinogen.

The most definitive evidence for linking diesel exhaust exposure to lung cancer comes from the Garshick et al. (1988) railroad worker study conducted in the United States. Relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 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 the risk of lung cancer increased with increasing duration of employment. As this was a 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, the observed association between increased lung cancer and exposure to diesel exhaust is more meaningful.

8.5.2. Case-Control Studies of Lung Cancer

Among the 10 lung cancer case-control studies reviewed in this chapter, only 2 studies did not find any increased risk of lung cancer. Lerchen et al. (1987) did not find any excess risk of lung cancer, after adjusting for age and smoking, for diesel fume exposure. The major limitation of this study was a lack of adequate exposure data derived from the job titles obtained from occupational histories. Next of kin provided the occupational histories for 50% of the cases that were not validated. The power of the study was small (analysis done on males only, 333 cases). Similarly, Boffeta et al. (1990) did not find any excess of lung cancer after adjusting for smoking and education. This study had a few methodological limitations. The lung cancer cases and controls were drawn from the ongoing study of tobacco-related diseases. It is interesting to note that the leading risk factor for lung cancer is cigarette smoking. The exposure was not measured. Instead, occupations were used as surrogates for exposure. Furthermore, there were very few individuals in the study who were exposed to diesel exhaust. 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) in workers who were exposed to diesel exhaust versus those 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% and 36%, respectively. Therefore, the positive results found in these studies are underestimated at best. In addition, the self-reported exposures used in 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 the inability to separate the exposures to diesel exhaust from those of gasoline exhaust because 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 between lung cancer and occupations with a 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% CI = 1.1, 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, because information on type of engine was lacking. Also, 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. Further, the methods used in these studies to classify occupational categories were different, probably leading to incompatibility of occupational categories.

The most convincing evidence comes from the Garshick et al. (1987) case-control study of railroad workers and the Steenland et al. (1990) case-control study of truck drivers in the Teamsters Union. Garshick et al. found that after adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95% CI = 1.05, 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), the relative odds increased to 1.43 (95% CI = 1.06, 1.94). This study appears to be a 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.

Steenland et al. (1990), on the other hand, created two separate work history files, one from Teamsters Union pension files and the other from next-of-kin interviews. Using duration of employment as a categorical variable and considering employment after 1959 (when presumed dieselization occurred) for long-haul drivers, the risk of lung cancer increased with increasing years of exposure. Using 1964 as the cutoff, a similar trend was observed for long-haul drivers. For short-haul drivers, the trend was positive with a 1959 cutoff but not when 1964 was used as the cutoff. For truck drivers who primarily drove diesel trucks and worked for 35 years, the relative odds were 1.89. The limitations of this study include possible misclassifications of exposure and smoking, lack of levels of diesel exposure, smaller nonexposed group, and insufficient latency period. Given these limitations, the findings of this study are probably underestimated.

Emmelin et al. (1993) in their Swedish dock workers from 15 ports found increased relative odds of 6.8 (90% CI = 1.3 to 34.9). Intricate exposure matrices were created using three different variables, but no direct exposure measurement was done. Of 50 cases and 154 controls, only 6 individuals were nonsmokers. A strong interaction between smoking and diesel exhaust was observed in this study.

8.5.3. Case-Control Studies of Bladder Cancer

Of the seven bladder cancer case-control studies, four 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% CI = 1.2, 349.5; Howe et al., 1980), truck drivers from New Hampshire and Vermont (OR = 2.9, $p<0.05$; Hoar and Hoover, 1985), and in Argentinean truck and railroad drivers (RR = 4.31, $p<0.002$; Iscovich et al., 1987). A positive trend with increasing employment as truck driver ($p=0.006$) was observed by Hoar and Hoover (1985) in their study of truck drivers from New Hampshire and Vermont. Significantly increased risks also were observed with increasing duration of employment of ≥20 years in truck drivers (OR = 12, $p=0.01$) and railroad workers (OR = 2.21, $p<0.05$; Steenland et al., 1987). No significant increased risk was found for any diesel-related occupations in studies by Wynder et al. (1985), Iyer et al. (1990), or Steineck et al. (1990). 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 (cigarette smoking, urinary retention, concentrated smoke within the truck cab, etc.); most of them had small sample sizes and none presented any latency analysis.

8.5.4. Relevant Methodologic Issues

Throughout this chapter various methodologic limitations of individual studies have been discussed, such as small sample size, short follow-up period, lack of latency analysis, and lack of data on confounding variables. However, two of the major methodologic concerns in these studies are use of death certificates to determine the cause of death and lack of data on cigarette smoking, which is a strong risk factor for both lung cancer and bladder cancer. Death certificates were used by all of the seven cohort mortality studies, two case-control studies of lung cancer, and one case-control study of bladder cancer to determine cause of death. Use of death certificates could lead to misclassification bias. Studies of autopsies done between 1960 and 1971 demonstrated that lung cancer was overdiagnosed when compared with hospital discharge, with no incidental cases found at autopsy (Rosenblatt et al., 1971). Schottenfeld et al. (1982) also found an overdiagnosis of lung cancer among autopsies conducted in 1977 and 1978. On the other hand, Percy et al. (1981) noted 95% concordance when comparing 10,000 lung cancer deaths observed in the Third National Cancer Survey from 1969 to 1971 (more than 90% were confirmed histologically) to death certificate-coded cause of death. For bladder cancer, the concordance rate was 91%. These more recent findings suggest that the diagnosis of lung cancer as well as bladder cancer on death certificates is better than anticipated. Furthermore, an overdiagnosis of lung cancer or bladder cancer on death certificates would reduce the ability of the study to detect an effect of diesel exhaust exposure.

All the cohort studies considered for this report are retrospective mortality studies. It is usually difficult to obtain smoking history in such instances. The smoking histories obtained

from surrogates (next of kin being either a spouse or an offspring) were found to be accurate by Lerchen and Samet (1986) and McLaughlin et al. (1987). Lerchen and Samet (1986) did not detect any consistent bias in the report of cigarette consumption. In contrast, overreporting of cigarette smoking by surrogates was observed by Rogot and Reid (1975), Kolonel et al. (1977), and Humble et al. (1984). Kolonel et al. (1977) found that the age at which an individual started smoking was reported within 4 years of actual age 84% of the time. The indication from these studies is that surrogates were able to provide fairly credible information on the smoking habits of the study subjects. If the surrogates of the cases were more likely to overreport cigarette smoking as compared to the controls, then it might be harder to find an effect of diesel exhaust because most of the increase in lung cancer would be attributed to smoking rather than to the effect of exposure to diesel exhaust.

8.5.5. Criteria of Causal Inference

In most situations epidemiologic data are used to delineate the causality of certain health effects. Several cancers have been causally associated with exposure to agents for which there is no direct biological evidence. Insufficient knowledge about the biological basis for diseases in humans makes it difficult to identify exposure to an agent as causal, particularly for malignant diseases when the exposure was in the distant past. Consequently, epidemiologists and biologists have provided a set of criteria that define a causal relationship between exposure and the health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually proves causality; actual proof is rarely attainable when dealing with environmental carcinogens. None of these criteria should be considered either necessary (except temporality of exposure) or sufficient in itself. The absence of any one or even several of these criteria does not prevent a causal interpretation. However, if more criteria apply, it provides more credible evidence for causality.

Thus, applying the criteria of causal inference to the seven cohort mortality and eight case-control studies in which risk of lung cancer was assessed resulted in the following:

- **Temporality:** There is a temporality of exposure to diesel exhaust prior to the occurrence of lung cancer.

- **Strength of association:** The strength of association between exposure and the occurrence of lung cancer in the cohort studies showed a 30% to 57% higher risk among exposed persons as compared to nonexposed (Howe et al., 1983; Wong et al., 1985; Boffetta and Stellman, 1988; Garshick et al., 1988). In case-control studies, the risk varied from 20% to 89% higher among exposed as compared to nonexposed (Williams et al., 1977; Hall and Wynder, 1984; Damber and Larsson, 1987; Garshick

et al., 1987; Benhamou et al., 1988; Hayes et al., 1989; Steenland et al., 1990; Gustavsson et al., 1990; Emmelin et al., 1993). Some of these studies did adjust for the confounding effects of smoking, asbestos, and other exposures. Furthermore, a recent publication by HEI (1995) demonstrates this strength of association in graphic presentation (Figures 8-1 and 8-2). Although the studies had smaller increases in lung cancer risk and only some of the studies considered by HEI (1995) are considered in this chapter, it demonstrates the lung cancer excesses consistently all across the various populations.

- **Consistency:** Five cohort studies and nine (including one nested case-control) case-control studies of lung cancer conducted in several populations in the United States and Europe consistently found the same effect (i.e., lung cancer).

- **Specificity:** All of the above-mentioned studies found the same specific effect (i.e., lung cancer).

- **Biological gradient:** The biological gradient, which refers to the dose-response relationship, was observed in the cohorts of Canadian railway workers (Howe et al., 1983), heavy bulldozer operators (Wong et al., 1985), and truck drivers who had enrolled in the American Cancer Society's prospective mortality study (Boffetta and Stellman, 1988). In the case-control studies, a dose response was observed in railroad workers (Garshick et al., 1988; Hayes et al., 1989; Steenland et al., 1990). Although other studies failed to observe a dose response, these studies were methodologically limited due to confounding by other exposures and lack of either quantitative data on exposure or surrogate data on dose.

- **Biological plausibility:** Because diesel exhaust consists of a carbon core particle with surface layers of organics and gases, the tumorigenic activity may reside in one, some, or all of these components. As explained in Chapter 9, there is clear evidence that the organic constituents have the capacity to interact with DNA and give rise to mutations, chromosomal aberrations, and cell transformations, all well-established steps in the process of carcinogenesis. Furthermore, these organic chemicals include a variety of polycyclic aromatic hydrocarbons and nitroaromatics, many of which are known to be pulmonary carcinogens. Alternatively, Vostal (1986) suggests that "diesel" particles themselves induce lung cancer, most likely via an epigenetic mechanism, if they are present at sufficiently high doses. This makes a convincing argument for biological plausibility of lung cancer occurrence under some condition of exposure.

When the same causal inference criteria were applied to the seven case-control studies in which risk of bladder cancer was assessed, the results were:

- **Temporality:** There is temporality of exposure to diesel exhaust prior to the occurrence of bladder cancer.

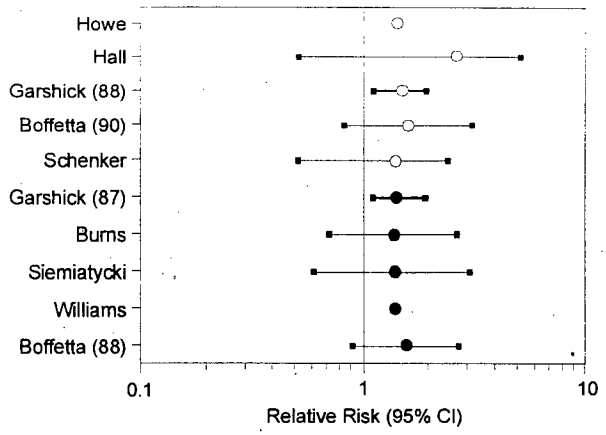

Figure 8-1. Lung cancer and exposure to diesel exhaust in railroad workers. ● = Relative risk adjusted for cigarette smoking; ○ = relative risk not adjusted for cigarette smoking. For the two studies by Howe and Williams, confidence intervals were not reported and could not be calculated.

Source: HEI, 1995.

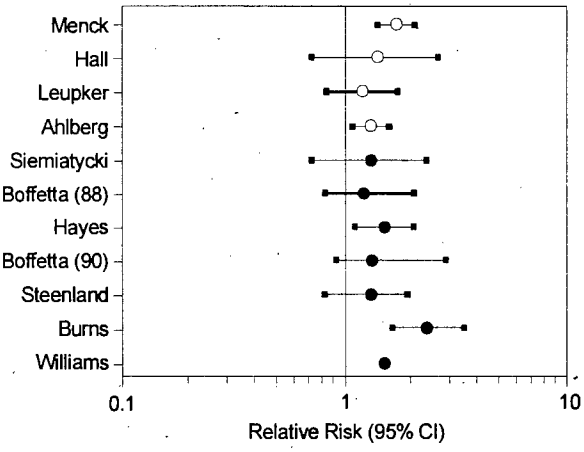

Figure 8-2. Lung cancer and exposure to diesel exhaust in truck drivers. ● = Relative risk adjusted for cigarette smoking; ○ = relative risk not adjusted for cigarette smoking. For the study by Williams, confidence intervals were not reported and could not be calulated. For the Steenland study, the data were gathered from union reports of long-haul truck drivers; for the Boffetta (1988) study, the data were self-reported by diesel truck drivers; and for the Siemiatycki study, they were self-reported by heavy-duty truck drivers (personal communication).

Source: HEI, 1995.

- **Strength of association:** The relative odds of getting bladder cancer among exposed as compared to nonexposed ranged from 2 to 12 times higher (Howe et al., 1980; Hoar and Hoover, 1985; Iscovich et al., 1987; Steenland et al., 1987). None of these studies adjusted for other confounding effects such as cigarette smoking, exposures to other chemicals, urinary retention, etc.

- **Consistency:** Four out of seven bladder case-control studies conducted in the United States and abroad found an increased relative odds of bladder cancer in the exposed population. None of the cohort studies showed increased bladder cancer mortality; however, people rarely die from bladder cancer, so bladder cancer excess is unlikely to be detected in mortality studies.

- **Specificity:** Four out of seven case-control studies found an excess of bladder cancer. The specificity criterion, per se, does not apply in this particular instance because these are case-control studies.

- **Biological gradient:** Dose response was observed in two out of four studies showing increasing relative odds with increasing length of employment (Hoar and Hoover, 1985; Steenland et al., 1987).

- **Biological plausibility:** It has been demonstrated that motor exhaust emissions contain PAHs and nitro-PAHs (Stenberg et al., 1983; Rosenkranz and Mermelstein, 1983). There is some evidence that nitro-PAHs may be responsible for the induction of human bladder cancer. Nitro-PAHs can be metabolized to aromatic amine derivatives, and some of these agents are known to be capable of inducing urinary bladder cancer (Clayson and Garner, 1976). Furthermore, 1-nitropyrene (1-NP) has been reported to be carcinogenic in the rat mammary gland (Hirose et al., 1984); the structurally related 4-aminobiphenyl, which induces bladder cancer in humans, also induces mammary gland tumors in rats (Hirose et al., 1984). Although the applicability of these experimental results to humans is unknown, the laboratory evidence certainly suggests the biological plausibility of diesel exhaust to be a urinary bladder carcinogen.

In summary, although some of the causality inference criteria do apply to bladder cancer, the evidence for bladder cancer in populations exposed to diesel exhaust is inadequate. On the other hand, all the causality inference criteria apply well to lung cancer. An excess risk of lung cancer was observed in four out of seven cohort studies and seven out of eight case-control studies. Dose response was found in three cohort studies and three case-control studies using duration of employment as a surrogate for dose. A recent meta-analysis (Bhatia, 1998) shows the consistency of elevated risks in 23 of 29 diesel exposure epidemiology studies, with statistically significant relative risks of 1.33. However, because of lack of actual data on exposure to diesel exhaust in these studies and other subtle methodologic limitations, the human

evidence falls just short of being sufficient to call diesel exhaust a human carcinogen. Using EPA's 1986 *Guidelines for Carcinogen Risk Assessment*, the human evidence alone is "limited," however, the "limited" classification doesn't indicate how close this is to being a known human carcinogen. Although diesel exhaust exposure is classified as "Limited," the human evidence is highly suggestive of a causal association between lung cancer and occupational exposure. Based on the human evidence alone, diesel exhaust is close to being a known human carcinogen.

8.6. REFERENCES

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9. MUTAGENICITY

Since 1978, more than 100 publications have appeared in which genotoxicity assays were used with diesel emissions, the volatile and particulate fractions (including extracts), or individual chemicals found in diesel emissions. Although most of the studies deal with the question of whether particulate extracts from diesel emissions possess mutagenic activity in microbial and mammalian cell assays, a number of 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 diesel particulate matter (DPM), and (4) exhaust filters or other modifications and other variables associated with diesel emissions. This chapter will focus on the application of the available data to issues of genetic risk assessment; reports dealing with mutagenic activity associated with the metabolism of particular chemicals of DPM are discussed in Chapter 10. Also, because of the large number of reports, this discussion will focus on key references. An International Agency for Research on Cancer (IARC) monograph (International Agency for Research on Cancer, 1989) contains an exhaustive description of the available studies and other review articles (Claxton, 1983; Pepelko and Peirano, 1983); the proceedings of several symposia on the health effects of diesel emissions (U.S. Environmental Protection Agency, 1980; Lewtas, 1982; Ishinishi et al., 1986; International Agency for Research on Cancer, 1989) are also available.

9.1. GENE MUTATIONS

Huisingh et al. (1978) demonstrated that dichloromethane extracts from DPM were mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and without rat liver S9 activation. This report contained data from several fractions as well as DPM from different vehicles and 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).

One approach to identifying significant mutagens in chemically complex environmental samples such as diesel exhaust or ambient particulate extracts is the combination of short-term bioassays with chemical fractionation (Scheutzle and Lewtas, 1986). The analysis is most frequently carried out by sequential extraction with increasingly polar or binary solvents. Prefractionation by silica-column chromatography separates compounds by polarity or into

acidic, basic, and neutral fractions. The resulting fractions are too complex to characterize by chemical methods, but the bioassay analysis can be used to determine fractions for further analysis. In most applications of this concept, *Salmonella* strain TA98 without the addition of S9 has been used as the indicator for mutagenic activity. Generally, a variety of nitrated polynuclear aromatic compounds have been found that account for a substantial portion of the mutagenicity (Liberti et al., 1984; Schuetzle and Frazer, 1986; Schuetzle and Perez, 1983). However, not all bacterial mutagenicity has been identified in this way, and the identity of the remainder of the mutagenic compounds remains unknown. The nitrated aromatics thus far identified in diesel exhaust were the subject of review in the IARC monograph on diesel exhaust (International Agency for Research on Cancer, 1989). In addition to the simple qualitative identification of mutagenic chemicals, several investigators have used numerical data to express mutagenic activity as activity per distance driven or mass of fuel consumed. These types of calculations have been the basis for estimates 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). However, as noted by Claxton (1983), because most of these studies used only strain TA98 without exogenous activation, several classes of mutagenic chemicals may have gone undetected.

Matsushita et al. (1986) tested particle-free diesel exhaust gas and a number of benzene nitro-derivatives and polycyclic aromatic hydrocarbons (PAHs) (many of which have been identified as components of diesel exhaust gas). The particle-free exhaust gas was positive in both TA100 and TA98, but only without S9 activation. Of the 94 nitrobenzene derivatives tested, 61 were mutagenic, and the majority showed greatest activity in TA100 without S9. Twenty-eight of 50 PAHs tested were mutagenic, all required the addition of S9 for detection, and most appeared to show a stronger response in TA100. When 1,6-dinitropyrene was mixed with various PAHs or an extract of heavy-duty (HD) diesel exhaust, the mutagenic activity in TA98 was greatly reduced when S9 was absent but was increased significantly when S9 was present. These latter results suggested that caution should be used in estimating mutagenicity (or other toxic effects) of complex mixtures from the specific activity of individual components.

Mitchell et al. (1981) reported mutagenic activity of DPM 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 Chinese hamster ovary (CHO) cells. Morimoto et al. (1986) used the APRT

and Oua^r loci in CHO cells; Curren et al. (1981) used Oua^r 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% of the observed activity.

Morimoto et al. (1986) injected DPM extracts (250 to 4,000 mg/kg) into pregnant Syrian hamsters and measured mutations at the APRT locus in embryo cells cultivated 11 days after i.p. injection. Neutral fractions from both light-duty (LD) and HD tar samples resulted in increased mutation frequency at 2,000 and 4,000 mg/kg. Belisario et al. (1984) applied the Ames test to urine from Sprague-Dawley rats exposed to single applications of DPM administered by gastric intubation, i.p. injection, or s.c. gelatin capsules. In all cases, dose-related increases were seen in TA98 (without and with S9) from urine concentrates taken 24 h after particle administration. Urine from Swiss mice exposed by inhalation to filtered exhaust (particle concentration 6 to 7 mg/m^3) for 7 weeks (Pereira et al., 1981a) or Fischer 344 rats exposed to DPM (2 mg/m^3) for 3 months to 2 years was negative in *Salmonella* strains. Because of the large differences in dosages, these findings should not be construed as conflicting.

Schuler and Niemeier (1981) exposed *Drosophila* males in a stainless steel chamber connected to the 3 m^3 chamber used for the chronic animal studies at EPA (see Hinners et al., 1980 for details). Flies were exposed for 8 h and mated to untreated females 2 days later. Although the frequency of sex-linked recessive lethals from treated males was not different from that of controls, the limited sample size precluded detecting less than a threefold increase over controls. The authors noted that, because there were no signs of toxicity, the flies might tolerate exposures to higher concentrations for longer time periods.

Specific-locus mutations were not induced in (C3H × 101)F_1 male mice exposed to diesel exhaust 8 h/day, 7 days/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.

9.2. CHROMOSOME EFFECTS

Mitchell et al. (1981) and Brooks et al. (1984) reported increases in sister chromatid exchanges (SCE) in CHO cells exposed to DPM extracts of emissions from both LD and HD diesel engines. Morimoto et al. (1986) observed increased SCE from both LD and HD DPM extracts in PAH-stimulated human lymphocyte cultures. Tucker et al. (1986) exposed human peripheral lymphocyte cultures from four donors to direct diesel exhaust for up to 3 h. Exhaust was cooled by pumping through a plastic tube about 20 feet long; airflow was 1.5 L/min. Samples were taken at 16, 48, and 160 min of exposure. Cell cycle delay was observed in all cultures; significantly increased SCE levels were reported for two of the four cultures. Structural chromosome aberrations were induced in CHO cells by DPM 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 8 h/day, 5 days/week for 1, 3, and 7 weeks. The incidence of micronuclei and structural aberrations was similar in bone marrow cells of both control and exposed mice. Increased incidences of micronuclei, but not SCE, were observed in bone marrow cells of male Chinese hamsters after 6 months of exposure to diesel exhaust (Pereira et al., 1981b).

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

Pereira et al. (1982) measured SCE in embryonic liver cells of Syrian hamsters. Pregnant females were exposed to diesel exhaust (containing about 12 mg/m^3 particles) from days 5 to 13 of gestation or injected intraperitoneally with diesel particles or particle extracts on gestational day 13 (18 h before sacrifice). Neither the incidence of SCE nor mitotic index was affected by exposure to diesel exhaust. The injection of DPM extracts but not DPM resulted in a dose-related increase in SCE; however, the toxicity of the DPM was about twofold greater than the DPM extract.

In the only studies with mammalian germ cells, 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 and mated to (SEC × C57BL/6) females, and the F_1 males were tested for

the presence of heritable translocations. Although no translocations were detected among 358 progeny tested, the historical control incidence is less than 1/1,000.

9.3. OTHER GENOTOXIC EFFECTS

Pereira et al. (1981b) exposed male strain A mice to diesel exhaust emissions for 31 or 39 weeks using the same exposure regimen noted in the previous section. Analyses of caudal sperm for sperm-head abnormalities were conducted independently in three separate laboratories. Although the incidence of sperm abnormalities was not significantly above controls in any of the three laboratories, there were extremely large differences in scoring among the three (control values were 9.2%, 14.9%, and 27.8% in the three laboratories). Conversely, male Chinese hamsters exposed for 6 mo (Pereira et al., 1981c) exhibited almost a threefold increase in sperm-head abnormalities. It is noted that the control incidence in the Chinese hamsters was less than 0.5%. Hence, it is not clear whether the differing responses reflect true species differences or experimental artifacts.

9.4. SUMMARY

Extensive studies with *Salmonella* have unequivocally demonstrated mutagenic activity in both particulate and gaseous fractions of diesel exhaust. In most of the studies using *Salmonella*, DPM extracts and individual nitropyrenes exhibited the strongest responses in strain TA98 when no exogenous activation was provided. Gaseous fractions reportedly showed greater response in TA100, whereas benzo[a]pyrene and other unsubstituted PAHs are mutagenic only in the presence of S9 fractions. The induction of gene mutations has been reported in several in vitro mammalian cell lines after exposure to extracts of DPM. Note that only the TK6 human cell line did not give a positive response to DPM extracts in the absence of S9 activation. Mutagenic activity was recovered in urine from animals treated with DPM by gastric intubation and i.p. and s.c. implants, but not by inhalation of DPM or diluted diesel exhaust. 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 particles and extracts. 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 a 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.

Mutagenicity data have been applied both to issues of heritable genetic risk and somatic cell effects, most notably cancer. For heritable genetic effects, the U.S. Environmental Protection Agency's Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1987) are applicable here. The mammalian germ-cell studies measuring defined genetic endpoints conducted on diesel emissions have shown negative results, but the sample size in the heritable translocation test is too small for a meaningful conclusion. In the absence of definitive mammalian germ-cell results, the guidelines recommend that mutagenic activity and the ability to interact with mammalian germ cells be evaluated separately. As stated, the presence of a large number of mutagenic chemicals in diesel emissions is unambiguous. Sperm abnormality assays are presumably the only other source of data on the interaction of diesel emissions with mammalian germ cells. The negative response in the mouse is in apparent conflict with the positive observation in the hamster, and there is not sufficient information to resolve this discrepancy. Hence, the questions of germ-cell interaction and the potential for human germ-cell mutagenic risk of diesel emissions remain unanswered.

The application of genotoxicity information to the question of the potential carcinogenicity of chemical agents was initially based on the premise that somatic mutation is an integral step in the carcinogenic process. However, unlike the situation for germ-cell mutagenicity, assays are not weighted strictly by their biological relationship to the particular species, sex, or tissue site of cancer. The size of the database and the degree of correlation of genotoxicity test results with animal cancer bioassays are frequently given great weight. Indeed, a common conclusion of the efforts of the National Toxicology Program on the use of in vitro assays is that no single in vitro genotoxicity test or battery of tests (among the four assays in their program) improves on the performance of the *Salmonella* assay in predicting rodent carcinogenicity of an untested chemical. When rodent carcinogenicity data are available, phylogenetic and other biological aspects of the genotoxicity data are important considerations in the weight-of-evidence process. With diesel emissions, additional complications arise because of the chemical complexity of the material being tested. Although it is clear that several of the individual chemical constituents of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic, it is likely that the constituents responsible for the mutational increases observed in bacteria are different from those responsible for the observed increases in CHO cells (Li and Dutcher, 1983) or in human hepatoma-derived cells (Eddy et al., 1986). Chapter 10 deals more thoroughly with metabolism and mechanisms of carcinogenesis.

9.5. REFERENCES

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

Considerable research has been directed toward assessing the carcinogenic potential of diesel engine emissions. As indicated in Chapter 7, whole diesel exhaust (DE) is a pulmonary carcinogen in rats subjected to chronic exposures at high concentrations. This response to date has been clearly demonstrated only in rats, and apparently involves particle overload resulting in inflammation and proliferation of alveolar epithelial cells and subsequent tumor formation. Studies assessing diesel exhaust-induced carcinogenicity in hamsters were negative, and studies in mice were equivocal (depending on the strain). The organic components that are adsorbed to the diesel exhaust particle do not appear to be required for expression of the high-dose tumorigenic response in rats. Positive responses are also observed following exposure to carbon black (CB) and TiO_2 particles that lack the organic components. The organic components, however, are likely to play a greater role in tumorigenic responses at lower exposure concentrations. In susceptible mouse strains and in humans exposed at low concentrations, genotoxic mechanisms induced by the organic components may even play a predominant role. Epidemiologic data suggest that there is a small increased cancer risk in humans following long-term occupational exposure to diesel exhaust. In examining mechanisms of diesel exhaust-induced carcinogenicity, it is necessary to address several areas, including (1) the carcinogenic potential of the carbon particle and the particle-overload effect, (2) the metabolism and mechanism of action of known carcinogenic components such as benzo[*a*]pyrene (B[*a*]P) and various nitroarenes, (3) the role of pulmonary leukocytes, and (4) DNA adduct formation in diesel exhaust exposures.

10.1. PARTICLE-INDUCED CARCINOGENIC RESPONSE

DE is a pulmonary carcinogen in rats chronically exposed to high concentrations (Heinrich et al., 1986; Mauderly et al., 1987). Additional studies (Vostal, 1986; Kawabata et al., 1986; Heinrich, 1990; Wolff et al., 1990; Oberdörster and Yu, 1990) provided data indicating that the carbonaceous core was a major factor in this response. In addition to diesel exhaust particulate matter (DPM), particle-specific pulmonary carcinogenesis in rats has been demonstrated for particles with virtually no adsorbed organics, such as CB (Heinrich et al., 1994, 1995; Nikula et al., 1995) and particles with no organic component, such as TiO_2 (Lee et al., 1985, 1986; Heinrich et al., 1995). Results of studies (see Chapter 7) from both the Inhalation Toxicology Research Institute (Nikula et al., 1995) and the Fraunhofer Institute of Toxicology and Aerosol Research (Heinrich et al., 1995) have affirmed the instrumental role of the carbon core in producing a carcinogenic response in rats chronically exposed to high concentrations (>2

mg/m^3) of whole diesel exhaust. The results of these studies clearly indicate that a particle overload effect is primarily responsible for this response. The rat alveolar epithelium may be predisposed to proliferative, metaplastic, and neoplastic responses, but the underlying mechanism for this effect is not clear. This section briefly reviews data affirming the particle effect, as well as data that provide some insight into possible mechanisms for this response.

A preliminary report by Heinrich (1990) provided evidence for a particle effect. In this study, female Wistar rats (72 per group) were exposed to Printex 90 CB particles for 10 mo followed by a 20-mo exposure-free observation period or for 20 months followed by a 10-mo exposure-free observation period. A particle concentration of 6.09 mg/m^3 was used in both protocols. The Printex 90 particles had an extremely low organic content (~1,000-fold less than that of DPM). The tumor rates for the 10- and 20-mo exposure durations were 17% (14% malignant) and 8% (all malignant), respectively. Although the lower tumor incidence for the longer exposure period was not consistent, the results demonstrate that the tumor incidences for CB particles with an organic content 1,000-fold less than DPM are equivalent to those reported for diesel exhaust exposures. The fact that these particles were able to exert a significant tumorigenic response implicates the carbon core of diesel exhaust particles as the possible tumor initiator in diesel exhaust-induced carcinogenicity at high particle concentrations.

More recently, an extensive study at the Fraunhofer Institute of Toxicology and Aerosol Research assessed the tumorigenic potential of DPM, the carbon core of DPM, CB, and TiO_2 in rats and two strains of mice (Heinrich et al., 1995). In this study, Wistar rats and NMRI mice were exposed to diesel engine whole exhaust, CB particles (Printex 90), and ultrafine TiO_2 particles for 2 years and to clean air for an additional 6 months. The results showed that when incidence of either all tumors or benign keratinizing cystic squamous-cell tumors was excluded, CB was at least as potent as DE when lung particle burdens were comparable (see Chapter 7 for details). DE was more potent on the basis of administered dose. The latter relationship can be at least partially explained by the slower pulmonary clearance of DPM and suggests that DE may be more toxic to phagocytic cells responsible for particle clearance.

Research efforts at the Inhalation Toxicology Research Institute (ITRI) have also provided data regarding the carcinogenic potential of whole diesel exhaust and of CB particles. In a long-term study, rats were exposed 16 h/day, 5 days/week for 24 mo to whole DE or CB (free of adsorbed organics) at particle concentrations of 2.5 or 6.5 mg/m^3 (Mauderly et al., 1991; Nikula et al., 1995). Controls were exposed to clean air. Although the CB particles were not totally devoid of organic components, the solvent-extractable fraction was small and the CB mutagenicity per unit of particle mass was three orders of magnitude less than that of diesel exhaust soot. Lung weights were increased in rats exposed to the highest concentrations of both diesel exhaust or CB but were slightly higher for the diesel exhaust group. The lung burdens of

particulate matter were significantly greater for the diesel exhaust-exposed rats at 18 and 23 mo. A substantial transfer of particles from the lungs to lung-associated lymph nodes was observed, but no difference was noted between the diesel exhaust and CB exposure groups. Inflammation and cytotoxicity detected in lavage fluid were greater for diesel exhaust-exposed rats, but the difference was proportional to the higher lung burden of retained particles noted for these animals. Tumor incidences and prevalence were similar for diesel soot-exposed and CB-exposed rats. Tumor types observed included squamous cysts, squamous cell carcinomas, papillary adenocarcinomas, tubular adenocarcinomas, and solid carcinomas. The growth of tumors transplanted into athymic mice also has been similar for diesel exhaust and CB exposures (74% and 73%, respectively).

Additional support for the particle-overload effect has been provided by data showing similar lung tumor rates in rats following intratracheal instillation of diesel exhaust soot or CB particles (Pott et al., 1994). Total primary lung tumors (expressed as percent and including adenomas, adenocarcinomas, cystic keratinizing squamous cell tumors, and squamous cell carcinomas) for the three DE groups were 65%, 60%, and 66%, whereas total tumors for the CB group were 65%. In addition to similar tumor rates, tumors induced by DPM and CB exhibited similar histopathologic patterns. Finally, Kawabata et al. (1993) induced lung tumors in rats intratracheally instilled with DPM from which the organic components had been extracted.

Wolff et al. (1990) reported the results of a study comparing pulmonary inflammation and DNA adduct formation in rats exposed 7 h/day, 5 days/week for 12 weeks to diesel exhaust or CB at concentrations of 10 mg/m^3. Although the level of lung DNA adducts was slightly higher for DE exposure, both exposures resulted in inflammatory responses, as determined by increased numbers of neutrophils and macrophages and increased acid proteinase in the bronchoalveolar lavage fluid.

Oberdörster and Yu (1990) evaluated the relationship between tumorigenic response of the lung and physical characteristics of various insoluble particles. Based on data from studies examining the effects of long-term inhalation exposure to DE TiO_2 particles, CB, or toner particles, they found that only the surface area of retained particles in the lung showed a reasonable concentration-response relationship relative to tumor incidence. Based upon this information, particle overload (retained mass or volume of particles) may not be the only determining factor in lung tumor formation for insoluble particles. The investigators hypothesized that a tumorigenic effect would probably require that a "critical" surface area of retained particles be attained for the manifestation of any mechanisms of tumorigenicity.

In comparing the potency of DE and CB, therefore, the particle surface area requires consideration. The Printex particles used in the study by Heinrich et al. (1995) have a large surface area (≈227 m^2/g), whereas the surface area of DPM varies from 18 (unextracted organics)

to 107 m^2/g (organics fully extracted). Although the actual mean surface area of DPM in the lungs is uncertain, it is less than that of the Printex particles. It could be hypothesized that particle effects from diesel exposure are less than those of CB because of a smaller specific surface, but the difference in potency is made up by surface-associated organics. Similar conclusions might also be derived from the intratracheal studies reported by Pott et al. (1994). Proof for this hypothesis may be difficult to achieve because surface area of the diesel particle would be expected to increase as organics are eluted from the particle surface during residence time in the lungs.

The involvement of persistent alveolar epithelial hyperplasia appears to be associated with the induction of neoplasia by particles. Oberdörster et al. (1995) reported an inflammatory influx of neutrophils in rats exposed for 3 mo to CB at concentrations of 7 or 50 mg/m^3 but not at 1 mg/m^3. Data showing exposure-response relationships for cytokine expression, neutrophilic inflammation, and dose-dependent alveolar Type II epithelial cell mutagenesis in rat lungs following 13-week exposure to CB (7 or 50 mg/m^3) were reported by Driscoll et al. (1995). Although these effects are seen at lower exposure concentrations as particle size decreases and specific surface area increases, the effects of ultrafine particles at less than particle overload conditions is uncertain.

In summary, results of these studies indicate that little difference exists in the type or incidence of lung tumors in rats following long-term exposure to DE or CB at high concentrations, that particle-associated organics are likely to play a minor role in lung particle overload-induced pulmonary carcinogenicity of DE in rats, and that particle effects are clearly relevant to the carcinogenic response observed. Persistent inflammation and hyperplasia of the alveolar epithelium, cytokine release, and release of oxidant reactants by alveolar macrophages (see Section 10.3) appear to be key elements leading to the species-specific neoplastic condition. The mechanistic basis for the surface-area-associated effects on cancer potency is as yet unknown.

10.2. METABOLISM AND MECHANISM OF ACTION OF ORGANIC CARCINOGENIC COMPONENTS OF DIESEL EXHAUST

DE is a complex mixture containing gaseous-phase components as well as soot particles to which more than 450 organic compounds are adsorbed (Opresko et al., 1984; Nikula et al., 1995). Although the involvement of particle-adsorbed organics in pulmonary carcinogenic responses appears to be minor in rats under particle overload conditions, the contribution of a subtle direct-mutagen effect should not be summarily dismissed, especially for humans.

In other species, such an effect may be more relevant, although currently available animal data and epidemiologic data do not support such a contention. A direct mutagen effect may be

inconsequential in rats relative to particle overload effects because bioavailability of the organic components is limited by the high-energy binding of these components with the carbon core. resulting in low desorption in the biological environment. The long residence times of particles in human lungs, however, may allow for greater desorption of organics.

The mechanism of action of many PAH carcinogens has been attributed to the reactivity of certain metabolic intermediates with cellular macromolecules and the subsequent formation of DNA adducts. The organics adsorbed to DPM may become available for biotransformation to known reactive intermediates, and macromolecular binding of these metabolites has been demonstrated.

Except for some of the DNA adduct studies, the available database does not allow a definitive discussion of the specific mechanism of carcinogenic action for these compounds relative to diesel exhaust specifically but rather is approached from the standpoint of the chemicals per se. Some of the data are derived primarily from in vitro studies that were not specifically concerned with the potential carcinogenicity of diesel exhaust but may be relevant because the compounds of concern are known components of diesel emissions.

More than 100 carcinogenic or potentially carcinogenic components have been specifically identified in diesel emissions, including various PAHs and nitroarenes such as 1-nitropyrene (1-NP) and dinitropyrenes (DNPs). These compounds are adsorbed to the carbon core of the particulate phase of the exhaust and, if desorbed, may become available for biological processes such as metabolic activation to mutagens. Among compounds identified from diesel exhaust are B[*a*]P, dibenz[*a,h*]anthracene, pyrene, chrysene, and nitroarenes such as 1-NP, 1,3-DNP, 1,6-DNP, and 1,8-DNP, 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 Stöber, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988).

There is evidence supporting a carcinogenic role for organics in the combustion process. Mumford et al. (1989) reported greatly increased lung cancer mortality in Chinese communes burning so-called "smoky coal," but not wood or smokeless coal, in unvented open-pit fires used for heating and cooking. Particle concentrations ranged from 10 to 25 mg/m^3 in communes burning either smoky coal or wood, but PAH levels were five to six times greater in the air of communes burning smoky coal. Thus cancer mortality correlated more closely with concentrations of PAHs than with particles. In the case of smokeless coal, both particle and PAH concentrations were low. Demonstration of the carcinogenicity of coke oven emissions in humans (Lloyd, 1971) also provided evidence for the role of organics, because coke oven particulate matter lacks an insoluble carbon core. It should be recognized, however, that PAH concentrations in these cases are much greater than can be expected from inhalation of DE.

Diesel particles may well enhance the activity of adsorbed organics. Adsorption of PAHs to a carrier particle such as hematite, CB, aluminum, or titanium dioxide enhances their carcinogenic potency (Farrell and Davis, 1974). In a more recent report, adsorption to carbon particles greatly enhanced the tumorigenicity of pyrolyzed pitch condensate containing B[*a*]P and other aromatic carcinogens (Heinrich et al., 1995). The increased effectiveness can be partly explained by more efficient transport to the deep lung. Slow release also enhances residence time in the lungs and prevents overwhelming of activating pathways. Even though the organic constituents may be tightly bound to the particle surface, clearance half-times of nearly 1 year in humans (Bohning et al., 1982) allow time for elution to occur. Furthermore, Gerde et al. (1991) presented a model demonstrating that large aggregrates of inert dust containing crystalline PAHs are unlikely to form at doses typical of human exposure. This allows the particles to deposit and react with the surrounding lung medium, without interference from other particles. Particle-associated PAHs can then be expected to be released more rapidly from the particles. Bond et al. (1984) provided evidence that alveolar macrophages from beagle dogs metabolized B[*a*]P coated on diesel particles to proximate carcinogenic forms. Unless present on the particle surface, B[*a*]P is more likely to pass directly into the bloodstream and escape activation by phagocytic cells.

The importance of DE-associated PAHs in the induction of lung cancer in humans may be enhanced because of the possibility that the human lung is more sensitive to these compounds than rat lungs. Rosenkranz (1996) summarized information indicating that in humans and mice, large proportions of lung cancers contain both mutated *p*53 suppressor genes and K-*ras* genes. Induction of mutations in these genes by genotoxins, however, is much lower in rats than in humans or mice. It could be even be hypothesized that lung tumors in diesel-exposed mice, which apparently could be induced only when the animals were exposed from conception to increase sensitivity (Pepelko and Peirano, 1983), may be due primarily to the organic fraction.

10.2.1. Metabolism and Disposition of B[*a*]P Relative to Diesel Exhaust

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 monooxygenases 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.

Relative to DE carcinogenicity, several studies have examined the metabolism and disposition of constituents such as B[*a*]P. Mitchell (1982) subjected 24 male F344 rats to nose-only inhalation of ^{3}H-B[*a*]P aerosol (500 mg/m^{3}) for 60 min. High levels of radiolabel were detected in the trachea, lungs, and turbinates. Based on measurement of the radiolabel, biphasic clearance was noted with half-time ($t_{1/2}$) values of 2 to 3 h and 25 to 56 h. Absorption by the lungs and systemic distribution were 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 days, 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 and potential metabolites following inhalation exposure.

Metabolization of intratracheally instilled B[*a*]P (1.0 µg) in strain A/J mice exposed to diluted diesel exhaust (8 h/day, 7 days/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 et al., 1980) examined the effects of prior DE 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. High-performance liquid chromatography (HPLC) analysis detected free B[*a*]P and nonconjugated primary metabolites and sulfate, glucuronide, and glutathione conjugates in each of the tissues. The occurrence of primary and secondary B[*a*]P metabolites in all three tissues was verified. The major hepatic metabolite was 3-hydroxy-B[*a*]P. The investigators concluded that diesel exhaust exposure may qualitatively affect the metabolism of B[*a*]P but does not significantly affect its distribution.

Sun et al. (1984) provided additional information comparing the disposition of particle-adsorbed B[*a*]P (0.1 wt %) and pure B[*a*]P following 30 min of nose-only inhalation by F344 rats. Long-term lung retention (percentage retained after 7 days) 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 days, the latter representing clearance of 50% of the initially deposited radiolabel. Clearance of pure B[*a*]P aerosol was >99% within 2 h and was apparently caused by 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% to 76% was B[*a*]P, 13% to 17% was B[*a*]P-phenol, and 5% to 18% was B[*a*]P-quinone. Although the Sun et al. (1984) study demonstrated the 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., 1986; Wood et al., 1976).

Leung et al. (1988) studied the role of microsomes in the removal and metabolism of B[*a*]P from DPM. Hepatic and lung microsomal preparations were made from 3-methylcholanthrene-induced F344 rats. ^{14}Carbon-B[*a*]P was adsorbed to DPM (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 these modified exhaust particles and that this capacity was dependent on the lipid content of the microsomes. Only small (<3%) amounts of B[*a*]P were transferred from the particles, with only 1% to 2% of this being metabolized. Free B[*a*]P, however, was extensively metabolized by the microsomes to B[*a*]P-9-10-diol. Relative to the liver microsomes, the lung microsomes exhibited an approximate twofold greater efficiency in the transfer of particle-associated B[*a*]P.

Bond et al. (1984) demonstrated metabolism of particle-associated B[*a*]P and free B[*a*]P by alveolar macrophages (AMs). 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 quinones were detected in both the cells and the media. The total amount of metabolites from both the cells and media increased with increasing incubation time up to 48 h. However, use of B[*a*]P in solution or B[*a*]P coated onto DPM did not alter the total amount of metabolites produced by the macrophages over a 24-h incubation period.

Because macrophages are instrumental in sequestering and transporting DPM matter in the lungs, AM-mediated metabolism of particle-associated B[*a*]P has been studied. Although some data show the ability of the AMs to metabolize B[*a*]P associated with DPM, Chen and Vostal (1982) have reported that aryl hydrocarbon hydroxylase (AHH) in AMs is decreased after in vivo exposure to diesel exhaust. Whether such diesel-associated decreases in AM enzymatic activity are counterbalanced by increases in the AM population size in response to diesel particle deposition (White and Garg, 1981) is unknown. Although it is known that human AMs contain AHH activity (McLemore et al., 1981) and that they can metabolize B[*a*]P (Harris, 1985), comparative studies of the AHH activities in rat, hamster, and human AMs could contribute toward determining the relationship such activity may have on the development of lung tumors.

Even though the AMs appear to contain the bulk of diesel particles deposited in the lung during chronic exposures, other cell types may also participate in the sequestration and/or metabolic activation of carcinogenic agents. The ability of lung epithelial cells to sequester diesel exhaust particles was reported by White and Garg (1981). Furthermore, significant metabolism of B[*a*]P by rat Type II alveolar 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% of the total B[*a*]P metabolites. Compared with the AMs that were examined in the aforementioned study, the rat Type II cells showed approximately 10 times greater ability to metabolize B[*a*]P.

Under healthy conditions, the Type II cells represent about 12% to 16% of all cells in the pulmonary epithelium of mammalian lungs and account for approximately 4% to 9% of the cells in the lungs (Crapo et al., 1983). Alveolar macrophages, on the other hand, account for approximately 4% to 9% of the cells in the pulmonary region (Crapo et al., 1983). In terms of their relative abilities to metabolize B[*a*]P, the Type II cells may play an even more important role than the AMs in metabolically activating PAH, assuming PAH as a substrate is available to them (e.g., extraction of PAH from diesel particles by AMs and the subsequent release of PAH or metabolically susceptible metabolites of PAH at Type II cell sites). The Type II cell hyperplasia observed after the deposition of diesel 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 important in the metabolism of PAH to ultimate carcinogens is the nonciliated bronchiolar cell. These cells are relatively rich in chemical metabolizing enzymes and, being also in a region of the respiratory tract where clearance of material would be relatively fast, may receive exposure via mucus to organics that have desorbed in the pulmonary region. The respiratory tract cytochrome 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 nonciliated bronchiolar cell (Boyd, 1984). It is worthy to note that bronchoalveolar adenomas that develop following diesel exposure have been found to resemble both Type II and nonciliated bronchiolar cells (Mauderly et al., 1987). Like the Type II cells, the nonciliated bronchiolar cells are not especially important in phagocytosis of particles deposited in the lung, although some particles may enter these cells. As previously indicated, any metabolism of procarcinogens by these cells probably involves the preextraction of carcinogen(s) in the extracellular lining fluid and/or in other endocytic cells.

Although the preceding studies indicate that particle-adsorbed B[*a*]P may be distributed throughout much of the organism via absorption from the lung and transport by the mucociliary escalator to the gastrointestinal tract, it is imperative to note that particles containing additional deposits of B[*a*]P exhibit greater potential for elution of organics than is observed for actual DPM. Therefore, the dissociation rates for the exhaust particles containing thermally deposited B[*a*]P do not realistically represent the dissociation of combustion-adsorbed organics. Even though particle-associated B[*a*]P can ultimately be metabolized by AMs and/or Type II cells to reactive intermediates, the contribution of this process to carcinogenic potential is uncertain and,

in rats, is probably of questionable significance. The relevant importance may be different in humans, however, where particle clearance rates have half-times of ≈ 1 year, thereby allowing greater time for elution of organics.

10.2.2. Metabolism and Disposition of Nitroarenes

Diesel engine emissions contain a large number of components, including numerous nitroarenes. Quantitatively, the nitroarenes represent a relatively small contribution to the overall PAH component of diesel engine emissions. However, with respect to carcinogenic potential, some of the nitroarenes (e.g., 1-NP, 4-NP, 6-nitrochrysene, and some DNPs) are of concern because of their known or suspected carcinogenic activity and their high mutagenic activity in some test systems (Manabe et al., 1985; International Agency for Research on Cancer, 1989). Within the scope of this document, it is inappropriate to review all of the studies regarding the carcinogenicity, metabolism, and mechanism of action of these various nitroarenes. Therefore, emphasis has been placed on those nitroarenes considered by the International Agency for Research on Cancer (1989).

1-Nitropyrene, a genotoxic and carcinogenic nitrosubstituted organic, 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 DPM.

Bond and Mauderly (1984) made quantitative measurements of 1-NP metabolism and macromolecular covalent binding in the isolated perfused rat lung. The study verified oxidation, reduction, acetylation, and conjugation biotransformation of 1-NP by the lung, with oxidation being the major process. The major metabolites were 3-, 6-, and 8-hydroxynitropyrene. The overall metabolism of 1-NP was increased by prior exposure of the rats to the mixed-function oxygenase (MFO) inducer 3-methylcholanthrene (3-MC) but not to phenobarbital. This 3-MC-induced increase in 1-NP metabolism and a parallel increase in macromolecular covalent binding suggest that this pathway may be responsible for the observed covalent binding.

Exposure of rats to diesel exhaust (7.4 mg/m^3) for 7 h/day, 5 days/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 among lower exposure (0.35 or 3.3 mg/m^3) groups or clean air controls. The data from this study indicate that exposure to DPM matter at concentrations of 7.4 mg/m^3 significantly alters the metabolism and subsequent covalent binding of nitropyrene.

Bond et al. (1986) also 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 DPM. Pure 1-NP was more efficiently absorbed in the lung than was 1-NP coated onto DPM 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 extrapulmonary 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 DPM may be significant relative to the dose to the lungs of both parent compound and metabolites.

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). More than 50% of the radiolabel was recovered (20% to 30% in the urine and 40% to 60% in the feces) within 24 h, regardless of the route of administration. The metabolic profile and elimination kinetics were similar for all routes of administration. The principal urinary metabolite (representing 15% to 25% 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, for the observed enterohepatic circulation of metabolites excreted in the bile, and for excretion of mutagenic activity in the urine. That intestinal microorganisms may alter the metabolites of 1-NP and facilitate their reabsorption was also reported by Medinsky et al. (1985). Accumulation of ^{14}C and diesel exhaust particles was detected in the lungs and gastrointestinal tract 24 h after intratracheal administration, 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 macromolecules, the authors note the possible risk to the gastrointestinal tract and lungs relative to 1-NP.

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, dichloromethane extract of DPM, or 1-NP. The results indicated that 1-NP was 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. Benzo[*a*]pyrene was not as potent a binding agent. Preexposure to the DPM extract but not to B[*a*]P resulted in increased DNA binding of B[*a*]P. Pretreatment with the dichloromethane

extract of DPM failed to increase the DNA binding of the nitropyrenes. The significance of this report is the demonstration that exposure to DE may potentiate the DNA binding of some of its components.

The preceding studies have shown that some of the nitroarenes known to be constituents of DE may undergo biotransformation to various metabolites, some of which are known to be carcinogenic to animal species. Such data may become more relevant as a complete understanding is obtained regarding the desorption of these compounds from DPM and their subsequent availability for biotransformation processes.

Nitroarenes quantitatively represent a relatively small portion of the PAH component of DE and, at least in rats, may play a minor role in tumorigenic responses compared to the particle overload effect. However, their contribution to the potential carcinogenicity of diesel engine emissions deserves some consideration. In the previous section, information was presented regarding the in vivo and in vitro metabolism of various nitroarenes considered to be possible human carcinogens. The fact that some of these metabolites have been shown to form DNA adducts in animal studies and are mutagenic in several test systems warrants their inclusion in assessing possible mechanisms of diesel-exhaust-induced carcinogenicity. In fact, Gallagher et al. (1994) reported results suggesting that DNA adducts are formed from nitro-PAHs present in DE and may play a role in the carcinogenic process.

However, the question remains as to why animals exposed to DE do not develop tumors characteristic of those induced by dinitropyrene. Rosenkranz (1995, 1996) provided evidence in support of a hypothesis that highly carcinogenic dinitropyrenes present on DE are bioactivated only at low exposure concentrations. Specifically, diesel exhaust exposure-mediated oxidative stress may prevent the reduction of dinitropyrenes to arylhydroxylamines and the subsequent formation of reactive arylnitrenium species that form ^{8}C-DNA adducts. Such an oxidative stress may also result in the oxidation of the arylhydroxylamines to nitrosoarenes that are incapable of reacting with DNA (Boldt et al., 1991). Additionally, the inflammatory response resulting from particle overload also may induce oxidative stress-like conditions. This hypothesis suggests that there are other potential carcinogenic mechanisms that could be expressed in other species or under different exposure conditions.

10.2.3. Formation of Reactive Oxygen Species From Organic Constituents of Diesel Exhaust and Their Involvement in the Induction of Lung Cancer

Sagai et al. (1993) reported that diesel exhaust particulate matter (DEP) could produce superoxide and hydroxyl radicals without any biological activating systems. DEP washed with methanol could no longer produce these radicals, indicating that the active components were extractable with organic solvents. The likely involvement of reactive oxygen species in the

induction of toxic and potentially carcinogenic effects in the lungs was suggested by greatly reduced lung injury due to DEP following extraction of the organic fraction prior to intratracheal instillation in mice. Additional support for the involvement of radicals in tissue damage was also provided by the finding that pretreatment with superoxide dismutase (SOD), an antioxidant, markedly reduced lung injury and death due to instillation of DEP. Similarly Hirafuji et al. (1995) found that catalase, deferoxamine, and MK-447 inhibited the toxic effects of DEP on guinea pig tracheal cells and tissues in vitro. Nagashima et al. (1995) demonstrated that the production of 8-hydroxydeoxyguanosine (8-OHdG), a product of the reaction of guanine with oxygen radicals, is induced in mouse lungs by intratracheal instillation of DEP. Ichinose et al. (1997) reported further that while intratracheal instillation of washed DEP in mice induced a small but statistically significant increase in lung tumor incidence, unwashed DEP induced a larger increase. Moreover, increases in 8-OHdG correlated very well with increases in tumor rates. It thus appears likely that generation of reactive oxygen species resulting from exposure to DEP may be involved in the carcinogenic process. It should be noted, however, that this nucleoside is efficiently repaired and that proof of a causal relationship in humans is still lacking. It is also unlikely that superoxide or hydroxyl radicals chemically generated by DEP alone promote 8-OHdG production in vivo and induce lung toxicity, because SOD is extensively located in mammalian tissues. A recent study indicates that the major route by which active oxygen species are generated from DEP components is via the P450 reductase system (Kumagai et al., 1997).

10.3. 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, at least in part, result from these cells' ability to produce a tumor necrosis factor (TNF) (Urban et al., 1986). Whether the tumor surveillance and tumoricidal activities of AMs (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. The possibility remains that diesel and other types of particles at high lung burdens result in decreases in natural killer (NK) cell functional activities in providing defense against tumor formation, either by direct particle-cell interactions or by altering the ability of AMs to influence NK cell-mediated host defense against metastatic tumor cells (Sone, 1986). These cells are subpopulations of lymphocytes that possess spontaneous cytolytic activity toward neoplastic cells but not toward normal cells. 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.

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. Hatch and co-workers (1980) have demonstrated that interactions of guinea pig AMs with a wide variety of particles, such as silica, metal oxide-coated fly ash, polymethylmethacrylate beads, chrysotile asbestos, fugitive dusts, polybead carboxylate microspheres, glass and latex beads, uncoated fly ash, and fiberglass increase the production of reactive oxygen species. Similar findings have been reported by numerous investigators for human, rabbit, mouse, and guinea pig AMs (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). As well, polymorphonuclear leukocytes (PMNs) are also known to increase production of superoxide radicals, hydrogen peroxide, and hydroxyl radicals in response to membrane-reactive agents and particles (Goldstein et al., 1975; Weiss et al., 1978; Root and Metcalf, 1977).

It is well recognized that the deposition of particles in the lung can result in the efflux of PMNs from the vascular compartment into the alveolar space compartment in addition to expanding the AM population size. Following acute exposures, the influx of the PMNs is transient, lasting only a few days (Adamson and Bowden, 1978; Bowden and Adamson, 1978; Lehnert et al., 1988). Strom (1984) has reported that PMNs become abnormally abundant following chronic exposures to DE. In the study by Strom (1984), the numbers of PMNs lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled DPM concentration. Strom (1984) also found that PMNs in diesel-exposed lungs remained persistently elevated for at least 4 mo after cessation of exposure, a potential mechanism that may be related to an ongoing release of previously phagocytized particles by AMs that engulfed them shortly after deposition. Evidence in support of this possibility has been obtained by Lehnert et al. (1989) in a study in which rats were intratracheally instilled with 0.85, 1.06, or 3.6 mg of polystyrene particles. The PMNs 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 PMNs became progressively greater over time. Such findings are consistent with an ongoing particle relapse process, given the relatively short lifespan of PMNs. As previously indicated, lung tumors develop in the rat at lesser lung burdens of DPM than with particles such as TiO_2. Polymorphonuclear leukocytes characteristically are increased abnormally in the lung by DE 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 AMs and PMNs 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 deposition of DPM.

As previously indicated, the production of oxygen species may afford protection against emerging tumor cells by killing the cells, whereas under other conditions the production of reactive oxygen products conceivably may contribute to the development of neoplastic cells. The potential involvement of AMs and PMNs in the development of lung tumors in laboratory rats administered high lung burdens of DPM (Mauderly et al., 1987) or having inhaled particles that are generally considered to have low to no cytotoxic potential (e.g., TiO_2 [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). Even though products of phagocytic oxidative metabolism, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, 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 to 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 PMNs and blood monocytes; blood lymphocytes alone were not mutagenic. These investigators speculated that the mutagenic activity of the phagocytes was a result of their ability to produce reactive oxygen metabolites, inasmuch as blood leukocytes from a patient with chronic granulomatous diseases, 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, 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).

Another characteristic of AMs and PMNs that may contribute to the pathologic process leading to lung tumor development following DPM deposition is that these phagocytes are known to release a variety of potentially destructive hydrolytic enzymes, a process 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 to Type I cells. Evans et al. (1986)

showed that injury to Type I cells is followed shortly thereafter by a proliferation of Type II cells. 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 unreactive polystyrene microspheres. Exaggerated proliferation as a repair or defensive response to DPM 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 DPM 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 their products 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 cell damage. Alveolar macrophages 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). The AM-derived mediators that may stimulate Type II cell hyperplasia following particle deposition in the lung remain to be identified, if in fact the AMs play a regulatory role in the Type II cell proliferative response.

Driscoll (1995) and Oberdörster and Yu (1991) outlined a proposed mechanism for the carcinogenicity of DE at high doses that emphasizes the role of phagocytic cells. Following exposure, phagocytosis of particles acts as a stimulant for oxidant production and inflammatory cytokine release by lung phagocytes. It was hypothesized that at high particle exposure concentrations the quantity of mediators released by particle-stimulated phagocytes exceeds the inflammatory defenses of the lung (e.g., antioxidants, oxidant metabolizing enzymes, protease inhibitors, cytokine inhibitors, etc.), resulting in tissue injury and inflammation. With continued particle exposure and/or the persistence of excessive particle burdens, there then develops an environment of phagocytic activation, excessive mediator release-tissue injury and, consequently, more tissue injury, inflammation, and tissue release. This is accompanied by cell proliferation. As discussed in a review by Cohen and Ellwein (1991), conceptually cell proliferation can increase the likelihood that any oxidant-induced or spontaneously occurring genetic damage becomes fixed in a dividing cell and is clonally expanded. The net result of chronic particle exposures sufficient to elicit inflammation and cell proliferation in the rat lung is an increased probability that the genetic changes necessary for neoplastic transformation will occur. In support of this hypothesis, it was reported that concentrations of inhaled CB resulting in lung inflammation increased mutation rates, an effect ameliorated by treatment with antioxidants

(Driscoll, 1995). Although these responses can occur in the absence of organic carcinogens, those present in DE may still contribute to the process.

The possibility that particles induce carcinogenic effects only at high doses still lacks conclusive proof. For example, it has not been definitively shown that inflammatory responses are a prerequisite for tumor induction. Furthermore, direct effects of ultrafine particles such as DPM taken up by epithelial cells cannot be ruled out. In fact, Riebe-Imre et al. (1994) reported that CB is taken up by lung epithelial cells in vitro, inducing chromosomal damage and disruption of the cytoskeleton, lesions that closely resemble those present in tumor cells. Johnson et al. (1993) reported that 20 nm polytetrafluoroethylene particles are taken up by pulmonary epithelial cells as well as polymorphonuclear leucocytes, inducing an approximate 4-, 8-, and 40-fold increase in the release of interleukin-1 alpha and beta, inducible nitric oxide synthetase, and macrophage inflammatory protein, respectively.

10.4. DNA ADDUCT CONSIDERATIONS

Although DNA adduct formation by particle-adsorbed organics was originally thought to be a mechanistic component of the diesel exhaust-induced pulmonary carcinogenesis observed in rats, currently available data do not support this hypothesis. The following section provides a brief overview of studies investigating DNA adduct formation following exposure to PAHs, DE, and other particulate matter.

On the assumption that DNA adduct formation is a critical step in the initiation of carcinogenesis (Harris, 1985), it was hypothesized that increased residence time of PAHs in the lung would increase the opportunity for metabolism and subsequent adduct formation. This would be especially important if association of the PAHs with soot particles and their slow release from those particles contributed to the increased residence time. Therefore, adduct formation by B[*a*]P alone compared with that of particle-associated B[*a*]P was investigated regarding possible mechanisms of diesel exhaust carcinogenicity.

An experiment was undertaken to test the hypothesis that inhalation of B[*a*]P associated with CB particles would increase the levels of DNA adducts compared with inhalation of pure B[*a*]P (Wolff et al., 1989). DNA modification was measured using the ^{32}P-postlabeling method developed by Randerath et al. (1985). The high sensitivity (~1 adduct in 10^{10} bases) of this technique (Reddy and Randerath, 1986) made possible 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 CB particles (97 mg/m^3) (B[*a*]P/CB), or filtered air. Total ^{14}C levels in the lung (a nonspecific indicator of reactive and nonreactive B[*a*]P metabolites, free B[*a*]P, and particle-bound B[*a*]P) 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 or the B[*a*]P diol-epoxide(BPDE)-DNA adduct in the lung were *not* significantly different whether the rats were exposed to pure B[*a*]P or B[*a*]P/CB. However, association of B[*a*]P with CB resulted in the formation of unidentified lung adducts that were not seen in DNA from lungs of rats exposed to pure B[*a*]P. It is possible that the adducts seen only in the B[*a*]P/CB exposures may play a role in the potential tumorigenic effect of particle-associated B[*a*]P. 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 from 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 adduct formation.

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 $t_{1/2}$ for the decline of total ^{14}C was approximately 10-fold faster 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 day 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 current data suggests that decline or repair of DNA adducts is considerably faster than that of protein turnover. Following repeated exposures, this would be expected to lead to increased buildup of ^{14}C in the lung relative to DNA adducts. The $t_{1/2}$ values for decline in DNA adducts observed in the current work are similar to the $t_{1/2}$ values 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 than the aforementioned $t_{1/2}$ values.

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 from 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 binding to DNA.

Caution should be used in interpreting the results from short-term exposures in regard to possible implications for long-term exposures when carcinogenicity might be observed. The pattern of results seen after 12 weeks 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, 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 is the possible influence of a chronic inflammatory

response, cell injury, or cell proliferation, all of which accompany long-term exposures to inhaled insoluble particles (Morrow, 1986). Such responses are generally greater after prolonged exposure than in the current 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 versus adsorbed onto the particle (Saffiotti et al., 1965).

Studies have also been conducted to evaluate DNA adduct formation in the lungs of animals exposed both to DE and to particles that are nearly devoid of organics (e.g., CB) or that completely lack organic fractions (e.g., TiO_2).

DNA adduct formation in the lungs of animals subjected to long-term exposure to whole DE has been described by Wong et al. (1986). Using tissues from animals of the Mauderly et al. (1987) study, these investigators reported an increase in DNA adduct formation in male and female F344 rats exposed to whole DE (7.1 mg of particles/m^3) for 7 h/day, 5 days/week for up to 30 mo. ^{32}P postlabeling was applied to DNA that was extracted from six control and six 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 airways and structures. Autoradiographic analysis revealed 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.

A study by Bond et al. (1989) addressed several key topics regarding the role of DNA adducts in the pulmonary carcinogenicity of DE. Using groups of rats exposed to whole DE at particle concentrations of 0, 0.35, 3.5, 7.0, or 10.0 mg/m^3 for 12 weeks, the relationship between DNA adduct levels and exposure concentration was examined. The data for the exposure levels employed indicated that DNA adduct formation (about 14 adducts per 10^9 bases) was similar across all exposure concentrations and was approximately twice that of the sham-exposed group. The fact that DNA adduct formation was independent of exposure concentration may be explained, in part, by previously reported data (Bond and Mauderly, 1984) showing that metabolism of organics associated with diesel exhaust by the isolated perfused rat lung could be saturated at high concentrations, thereby limiting the production of metabolites required for the formation of DNA adducts.

The time course for DNA adduct formation was also examined by Bond et al. (1989). Over a 12-week period of exposure to diesel exhaust (7 mg/m^3 DPM), lung DNA adducts were

found to accumulate slowly. The highest adduct level was reached at 12 weeks, followed by a decline to control level by 4 weeks postexposure. Throughout the exposure period, lung DNA adducts remained constant and at a lower level in sham-exposed rats. The investigators suggested that the rapid repair of adducts relative to their formation might result in a steady-state level of DNA adducts during long-term exposure.

A dosimetry study examined the distribution of DNA adducts in the respiratory tract to determine if increased DNA adduct formation occurred in regions of the lung where diesel exhaust-induced tumors are formed (Bond et al., 1988). For this study, rats were exposed for 12 weeks to DE at a particle concentration of 10 mg/m^3. DNA adduct levels were highest in peripheral tissue, which is the same region in which tumors occurred in rats in long-term exposure studies (Mauderly et al., 1987). Although these findings suggest that DNA adduct formation and tumor formation are related, the data do not prove the association.

The previous studies provided data regarding the role of DNA adducts in the pulmonary carcinogenesis of DE, but were not designed to provide insight into possible target cells. An additional molecular dosimetry study by Bond et al. (1990a) addressed this topic and also compared the effects of DPM with CB particles that were virtually free of the adsorbed organics found on DPM. In this study, rats were exposed to whole DE (6.2 mg/m^3), CB particles (6.2 mg/m^3 DPM), or clean air 16 h/day, 5 days/week for 12 weeks. Relative to clean air controls, a significant increase in the total DNA adduct level in Type II cells was noted for rats exposed to DE and CB. The exposure to CB and DE resulted in an approximate fourfold increase in adduct level compared with controls. However, the investigators noted that there was a large region of unresolved adducts in the chromatograms from DE-exposed rats and that the total adduct level in these animals may be underestimated. Whether the small amount (≈0.04%) of extractable organics on the CB particles was responsible for the observed DNA adduct formation or the adducts were the result of inflammatory responses to the particles was not determined. This study does, however, demonstrate that Type II cells are possible targets for diesel exhaust exposure.

The report by Bond et al. (1989) provided additional information to suggest a possible relationship between DNA adducts in the lung and DE-induced pulmonary carcinogenesis. For this study, rats, mice, hamsters, and monkeys were exposed to DE at a particle concentration of 8.1 mg/m^3 for 12 weeks. Following this exposure, the levels of lung DNA adducts in rats, a species susceptible to DE-induced carcinogenesis, were shown to be 60% greater than for sham-exposed controls. However, lung DNA adduct levels in mice and hamsters (species that have been shown to be resistant to exhaust-induced carcinogenesis) were very similar to those of respective controls. Also of interest was the finding that DNA adduct levels in the lungs of diesel exhaust-exposed monkeys were 80% greater than those of sham-exposed controls.

In study by Wolff et al. (1990) described earlier in this chapter, levels of DNA adducts as determined by ^{32}P postlabeling were significantly higher for DE-exposed rats than for CB-exposed rats after 12 weeks of exposure to DPM concentrations of 10 mg/m^3. The results of this study suggested that DNA adduct levels are influenced by the organic content of the carbonaceous particles and that the organic constituents may initiate carcinogenesis. It was hypothesized that the continued inflammatory and proliferative responses may then promote cell transformations. Although the DNA adduct formation may have been the result of very small amounts of organics desorbed from the carbon particles, it is also possible that these adducts are the result of oxygen radicals or other reactive agents released from neutrophils and macrophages. More recent reports have affirmed the latter contention. Randerath et al. (1992) and Williams et al. (1992) provided data showing DNA adduct levels to be increased following exposures to CB and that no organic fraction was involved.

Gallagher et al. (1994) noted that DNA adduct-like compounds were formed in rat lungs following exposures to DE, TiO_2, or CB, but that the total adduct levels were not significantly elevated by DE exposure. Exposure to DE resulted in DNA adducts, possibly from nitro-PAHs associated with the organic fraction of the DPM, but the overall significance of this mechanism in the carcinogenic response is uncertain.

The uncertainty of the role of a genotoxic mechanism was further shown by Swafford et al. (1995), who detected no differences in mutational patterns in CB-induced or diesel exhaust-induced pulmonary carcinomas. It was, however, noted that inactivation of the *p53* may have a role in neoplastic responses with a squamous-cell carcinoma component.

Based on the assumption that DNA adduct formation is a critical step in the initiation of carcinogenesis (Harris, 1985), increased residence time of PAHs in the lung would increase the opportunity for metabolism and subsequent adduct formation. Some involvement of the organic components is suggested by data showing the formation of DNA adducts in exposed animals and by the known carcinogenic and mutagenic potential of many of the compounds in 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, the fact that xenobiotics may undergo biotransformation to reactive intermediates following their entry into the body via inhalation of DPM has been demonstrated for B[*a*]P and various nitroarenes. However, results from the metabolism/disposition studies using carbon particles to which organics have been experimentally adsorbed must be interpreted with caution. The concentration of organics on these particles is probably much greater than the monomolecular or bimolecular layer on actual DPM and, therefore, might facilitate desorption of the organics from these experimentally prepared particles.

The role of DNA adducts in DE-induced pulmonary carcinogenesis requires further investigation (Bond, 1993). Because DNA adduct formation is observed after exposure to CB particles, formation of these adducts by particle-adsorbed organics does not appear to be a definitive mechanism for explaining the carcinogenic response observed in rats. However, a possible (albeit minor) contributory role for particle-adsorbed organics cannot be totally dismissed. Higher concentrations of CB than DPM were required to induce adduct formation, and Gallagher et al. (1994) reported a nuclease-sensitive adduct in rats exposed to DPM but not CB. It was hypothesized that this adduct may have resulted from exposure to nitro-PAHs.

Several investigators have reported on DNA adducts in humans exposed to diesel exhaust. In studying biomarkers of exposure, distinct adduct patterns were found among garage workers occupationally exposed to diesel exhaust when compared to nonexposed controls (Nielsen and Autrup, 1994). Furthermore, the findings were concordant with the adduct patterns observed in groups exposed to low concentrations of PAHs from combustion processes. Hemminki et al. (1994) also reported elevated levels of DNA adducts in lymphocytes from garage workers with known diesel exhaust exposure compared to nonexposed mechanics. The adduct levels (up to 3.63 adducts/10^8 nucleotides) were significantly greater in diesel exhaust-exposed groups when compared to nonexposed groups. Hou et al. (1995) found elevated adduct levels in bus maintenance workers exposed to diesel exhaust. Although no difference in mutant frequency was observed between the groups, the adduct levels were significantly different (3.2 vs. 2.3×10^{-8}). Nielsen et al. (1996) measured three biomarkers in DE-exposed bus garage workers: lymphocyte DNA adducts, hydroxyethylvaline adducts in hemoglobin, and 1-hydroxypyrene in urine. Significantly increased levels were reported for all three. Qu et al. (1996) detected increased adduct levels, as well as increases in some individual adducts, in the blood of underground coal miners exposed to DE.

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

Recent studies have shown tumor rates resulting from exposures to nearly organic-free CB particles to be similar to those observed for DE exposures, thus providing strong evidence for an epigenetic mechanism underlying DE-induced pulmonary carcinogenesis in rats at high doses. A nongenotoxic mechanism is also supported by the fact that carbon particles per se cause inflammatory responses and increased epithelial cell proliferation and that AM function may be compromised under conditions of particle overload. A mechanism was proposed in which particle-overloaded phagocytic cells secreted a variety of inflammatory mediators, stimulating cell proliferation and increasing the likelihood that any oxidant-induced or spontaneously occurring genetic damage would become fixed and clonally expanded, resulting in an increased

probability of neoplastic change. The development of lung tumors in rats following chronic exposures to diesel exhaust was detected under conditions in which AM-mediated particle clearance from the lung provides support for this hypothesis. It should be noted, however, that the bioassays lacked sensitivity to detect tumors at nonoverload conditions.

It is generally accepted that one of the underlying mechanisms of carcinogenesis involves the formation of covalent adducts with DNA, resulting in the alteration of cellular genetic information. Several reports have provided data indicating that such adducts are formed in animals after long-term exposure to DE. The premise that DNA adduct formation plays a role in DE-induced carcinogenesis is substantiated by several findings, including an increase in DNA adducts in the same pulmonary regions where tumors occur and higher DNA adduct levels in species known to be susceptible to DE-induced tumors. However, the lack of an exposure response for DNA adduct formation, as demonstrated by the molecular dosimetry studies reported by Bond et al. (1990b), suggests the involvement of additional mechanisms.

While particle overload mechanisms are likely to predominate at high exposure concentrations, organics are likely to play a greater role for any effects that may occur at non-overload exposure conditions. Although the lung's AMs, which phagocytize deposited DPM, may participate in the gradual in situ extraction and metabolism of procarcinogens associated with the diesel particles, it is uncertain if the mutagenic organics are or can be eluted in sufficient amounts to be relevant to a carcinogenic response in the rat model. The much slower particle clearance rates in humans, however, allow for greater efficiency of extraction. While both DE and CB have been reported to increase DNA adducts to a similar degree, DE is effective in non-particle-overload conditions, suggesting again that the organics may play a role in tumor induction. The participation of organics is supported by reports of increased DNA adducts in humans occupationally exposed to diesel exhaust (see Section 10.4).

Low-dose effects of DPM must also be considered, including the findings of Riebe-Imre et al. (1994), who showed that CB may induce chromosomal damage and cytoskeletal alterations in vitro at doses that are not cytotoxic. The role of reactive oxygen species must also be considered relative to low-dose DE exposure. The normal tumoricidal activities of the AMs 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. The in vitro formation of active oxygen radicals by DE exposure reported by Sagai et al. (1993) indicated that reactive oxygen species could be formed without bioactivation, and may thus occur even under nonoverload conditions. The in vivo induction of oxygen radical-induced DNA damage (Nagashima et al., 1995) provided additional support for low-dose particle effects.

Caution must be exercised in extrapolating observations made in animal models to humans when assessing the potential for DE-induced pulmonary carcinogenesis. The carcinogenic response and the formation of DNA adducts in rats exposed to diesel exhaust and other particles at high exposure concentrations may be species-specific and not particle-specific. However, DNA adduct data and the possible involvement of reactive oxygen species may be relevant in long-term occupational exposure of humans to low concentrations.

10.6. REFERENCES

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11. QUALITATIVE AND QUANTITATIVE EVALUATION OF THE CARCINOGENICITY OF DIESEL ENGINE EMISSIONS

11.1. INTRODUCTION

Concern about the carcinogenic risk of exposure to diesel engine emissions was stimulated in the late 1970s by a report indicating that diesel particle extracts are mutagenic (Huisingh et al., 1978), by the knowledge that diesel exhaust (DE) contains known carcinogens, and by the projected increase in the use of diesel engines in passenger vehicles. This concern culminated in a U.S. Environmental Protection Agency (EPA)-sponsored quantitative cancer risk estimate for diesel engine emissions (Albert et al., 1983). Its estimate was based on a "comparative potency" approach because of a lack of either chronic animal cancer bioassays or definitive epidemiologic data. Since 1983, several chronic animal inhalation studies and epidemiologic investigations designed to assess the carcinogenicity of diesel engine emissions have been completed. These studies are summarized in Chapters 7 and 8. Data relating to mechanisms of DE-induced carcinogenicity are summarized in Chapter 10. Because of the increase in the availability of such data and because of the need to provide an up-to-date evaluation of the hazards of DE inhalation for U.S. EPA's Office of Mobile Sources, a qualitative as well as a quantitative assessment of the cancer risk from exposure to DE was undertaken. These assessments are presented in this chapter.

11.2. QUALITATIVE EVALUATION OF THE CARCINOGENIC RISK OF EXPOSURE TO DIESEL EXHAUST

11.2.1. Hazard Evaluation Based on EPA 1986 Guidelines for Carcinogen Risk Assessment

Lung cancer incidence has been studied in human populations exposed to diesel engine exhaust. An increased incidence of lung cancer was observed in 6 mortality studies (Howe et al., 1983; Wong et al., 1985; Boffetta and Stellman, 1988; Garshick et al., 1988; Gustavsson et al., 1990; Ahlman et al., 1991) and in 10 case-control studies (Williams et al., 1977; Hall and Wynder, 1984; Damber and Larsson, 1987; Garshick et al., 1987; Benhamou et al., 1988; Hayes et al., 1989; Gustavsson et al., 1990; Steenland et al., 1990; Burns and Swanson, 1991; Emmelin et al., 1993). A dose-response trend was observed in three of the cohort studies (Howe et al., 1983; Wong et al., 1985; Boffetta and Stellman, 1988) and in three of the case-control studies (Garshick et al., 1987; Steenland et al., 1990; Emmelin et al., 1993). An increased incidence of lung cancer was not observed in some other studies (Waller, 1981; Rushton et al., 1983; Wong et al., 1985; Edling et al., 1987; Lerchen et al., 1987), but each had several methodologic limitations, such as small sample sizes, short follow-up, and lack of adjustment for confounding factors. The studies reporting increased incidences also had some major limitations even though

some of them, especially those reported by Garshick and co-workers (1987, 1988), were able to eliminate most of the confounding variables.

An association between DE exposure and lung cancer is therefore suggested by the results of a number of epidemiologic studies. Because of the uncertainties created by limited exposure data and the possibility of exposure to other agents, the evidence for carcinogenicity of diesel engine emissions in humans is considered to be limited under the EPA Cancer Assessment Guidelines (U.S. EPA, 1986).

In animal experiments, inhalation of whole DE resulted in the induction of lung tumors in F344 rats (Ishinishi et al., 1986; Iwai et al., 1986; Mauderly et al., 1987; Brightwell et al., 1989; Nikula et al., 1995), in Wistar rats (Heinrich et al., 1986a; Heinrich et al., 1995), in Sencar mice (Pepelko and Peirano, 1983), in NMRI mice (Heinrich et al., 1986a; Stöber, 1986), and in C57BL mice (Takemoto et al., 1986). It should be noted, however, that lung tumor incidences did not exceed those of historical controls in the 1986 NMRI mouse study, and in a later study reported by Heinrich et al. (1995), using both NMRI and C57BL mice, significant increases in lung tumors were not seen. Lung tumors also were induced by implantation of DE condensate in Osborne-Mendel rats (Grimmer et al., 1987) and by intratracheal instillation of diesel particulate matter in Wistar rats (Pott and Roller, 1994). Skin painting of diesel particle extracts induced dermal tumors in strain A mice (Kotin et al., 1955) and in Sencar mice following promotion with tetradecanoylphorbol-13-acetate (Nesnow et al., 1982). Extensive studies with *Salmonella* mutagenesis assays have, in most cases, demonstrated direct-acting mutagenic activity in both particle extracts and gaseous fractions of DE. Positive results also have been reported for gene mutations and chromosome effects in mammalian cell systems after exposure to diesel particle extracts.

Based on the induction of lung tumors via inhalation in at least two strains of rats and two strains of mice and by lung implantation of DE condensate, subcutaneous tumors following injection of exhaust particle organic extracts, and skin tumors following dermal application of exhaust particle organic extracts and supported by positive mutagenicity results, the evidence for carcinogenicity of DE in animals is considered to be sufficient under U.S. EPA's Cancer Assessment Guidelines (U.S. EPA, 1986).

The International Agency for Research on Cancer (IARC) (1989) also evaluated the evidence for carcinogenicity of DE and concluded that the evidence for carcinogenicity in humans is limited. This conclusion was based primarily on cohort studies of railroad workers in the United States (Garshick et al., 1988) and Canada (Howe et al., 1983) and two case-control studies (Howe et al., 1983; Garshick et al., 1988) using individuals drawn from the same group of workers. Three further studies of cohorts with less certain exposure to diesel engine exhaust also were considered: two studies of London bus company employees (Raffle, 1957; Rushton et

al., 1983) and one of Swedish dock workers (Edling et al., 1987). IARC concluded that the evidence for carcinogenicity of whole engine exhaust in experimental animals was adequate. The conclusions were based on positive tumorigenic effects in two different strains of rats in five of six experiments (Karagianes et al., 1981; Heinrich et al., 1986b; Ishinishi et al., 1986; Iwai et al., 1986; Mauderly et al., 1987) and on positive effects in mice in two studies (Pepelko and Peirano, 1983; Stöber, 1986).

The IARC conclusions are thus in general agreement with those of EPA. Both agencies concluded that human evidence is limited. IARC considered the animal evidence to be adequate for whole DE as well as for extracts of exhaust particles but inadequate for the gaseous phase. Although EPA has not specifically evaluated the gaseous phase and did not consider whole exhaust and particle extracts separately, both agencies agree that whole diesel engine exhaust is carcinogenic in animals.

On the basis of limited evidence for carcinogenicity in humans, diesel engine emissions are considered to best fit into the cancer weight-of-evidence Category B1, according to 1986 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 on diesel particles. Chemicals classified in Category B1 are considered to be probable human carcinogens. IARC (1989) also considers DE to be probably carcinogenic in humans and has therefore classified it as Category 2A.

11.2.2. Hazard Evaluation Based on EPA Proposed Guidelines for Carcinogen Risk Assessment

Under EPA's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), DE is considered to be a likely human carcinogen by the inhalation route of exposure. Its carcinogenic potential is indicated by limited evidence for carcinogenicity in occupationally exposed humans, positive carcinogenicity in laboratory animal studies, positive evidence for mutagenicity, and the presence of known human carcinogens in the mixture. In comparison with other agents designated as likely carcinogens, the overall weight of evidence for this agent places it at the upper end of this grouping. This is because of positive data on tumor responses in both humans and animals.

Concerning humans, increases in relative risks for lung cancer have been reported in a variety of populations occupationally exposed to DE. These increases have been small, generally less than 2. While the inability to eliminate all confounding variables increases uncertainty, most of the variables have been eliminated in at least one large study. Moreover, the reporting of increased relative risk in a number of studies with differing confounding factors increases the likelihood that a true response has occurred.

Concerning animals, clear evidence for induction of lung tumors in rats was reported in several well-designed and well-conducted experiments. Results in mice were equivocal with the only definitive response occurring in a sensitive strain of mice exposed from conception. Syrian hamsters did not show a positive response but are known to be resistant to induction of deep lung tumors. Experiments with cats, monkeys, and Chinese hamsters, while negative for tumor induction, were of insufficient exposure duration and/or exposure level.

In addition, DE contains a variety of compounds that are considered to be known or likely human carcinogens. These include several polycyclic aromatic compounds such as benzo[a]pyrene and nitroaromatics such a dintropyrene, 1,3-butadiene, and benzene. This evidence is limited because of the low concentration of most of these compounds and because of their uncertain bioavailability. Extracts of diesel exhaust particulate matter (DPM) are also genotoxic in most tests.

Biological information on DE is contradictory in terms of how to quantitate potential cancer risks. Among animal species, only rats have shown unequivocal tumor responses, raising the possibility that this response may be unique to rats. Tumor induction also was noted only at exposure concentrations resulting in lung particle overload and accompanying pathological effects. Because of limited sensitivity of the studies, however, the possibility of tumor induction at exposure levels below those responsible for particle overload still exists. Mechanistic studies support the concept that high-dose induction of lung cancer is a result of lung particle overload resulting in release of inflammatory cytokines, proteolytic enzymes, and reactive oxygen species, resulting in genotoxic effects with increased likelihood of fixing harmful mutations because of rapid cell turnover. On one hand, while epidemiologic data are limited because of small increases in relative risk ratios and an inability to eliminate all confounding factors, the data nevertheless suggest that humans are likely to respond with lung cancer under nonparticle-overload conditions. Low-dose effects are more likely due to elution of organics from the diesel particle, although direct effects of particles cannot be ruled out.

11.3. QUANTITATIVE ESTIMATION OF THE CARCINOGENIC RISK OF EXPOSURE TO DIESEL EXHAUST

11.3.1. Review of Previous Quantitative Risk Estimates

Early attempts to quantitatively assess the carcinogenicity of diesel engine emissions were hindered by a lack of both positive epidemiologic studies and long-term animal studies. One means of overcoming these obstacles was the use of the "comparative potency" method (Albert et al., 1983). A second involved estimating risk based on equivocal epidemiologic evidence (Harris, 1983).

In the comparative potency method, a combustion or pyrolysis product was selected that had a previously determined cancer potency estimate based on epidemiologic data. The ratios of the potency of this agent (e.g., coke oven emissions) to diesel exhaust particulate matter (DPM) extract in a variety of in vivo and in vitro 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 the 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 from 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. Dose-dependent increases in response were obtained for the four assays listed below:

- Ames *Salmonella typhimurium* (TA98) reverse mutation,
- Gene mutation in L5178Y mouse lymphoma cells,
- Sencar mouse skin tumor initiation test, and
- Viral enhancement of chemical transformation in Syrian hamster embryo cells.

Only the first three assays were used to develop comparative potency estimates because of variability of responses in the enhancement of the viral transformation assay. The in vitro studies were carried out both in the presence and absence 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 was positive for all the engines tested except the Caterpillar engine. Only the Nissan engine, however, gave strong dose-response data. Because skin tumor initiation was considered to be the most biologically relevant test, it was used to derive potency estimates for the Nissan engine. An estimate for the Nissan engine was then derived 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 engine. According to this method, three 95% upper-bound estimates of lifetime cancer risk per microgram per cubic meter of extractable organic matter were derived for the Nissan diesel, based on potency comparisons with each of the three agents. These values are: coke oven emissions, 2.6×10^{-4}; roofing tar, 5.2×10^{-4}; and cigarette smoke condensate, 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 TA98, the sister chromatid exchange assay in Chinese hamster ovary cells, and the mutation assay in mouse lymphoma cells. The estimated lifetime cancer risk per microgram per cubic meter of extractable organic matter for extracts from these engines are as follows: Volkswagen, 1.3×10^{-4}; Oldsmobile 1.2×10^{-4}; and Caterpillar, 6.6×10^{-6}.

To convert these values to a lifetime risk per microgram per cubic meter of total DPM, the results were multiplied by the fraction of extractable organic matter in the particles. This conversion was based on the assumption that the carcinogenic effects were caused solely by the organic fraction. These fractions were as follows: Nissan, 0.08; Volkswagen, 0.18; Oldsmobile, 0.17; and Caterpillar, 0.27. After this adjustment, the resulting estimated potencies per microgram per cubic meter of inhaled DPM varied from 3.5×10^{-5} for the Nissan to 1.8×10^{-6} for the Caterpillar.

Harris (1983) developed comparative potency estimates for the same four engines used by Albert et al. (1983) but used only two epidemiology-based potency estimates: those for coke oven emissions and for roofing tar. He employed preliminary data from three of the same assays used by Albert et al. (1983)—the Sencar mouse skin tumor initiation assay, enhancement of viral transformation in Syrian hamster embryo cells, and the L5178 mouse lymphoma test. The mouse lymphoma test was used both with and without metabolic activation, whereas the *Salmonella* assay was not used.

The diesel cancer potency estimates by Harris (1983) were then derived by multiplying the epidemiology-based cancer potency estimates for both coke oven emissions and roofing tar by the ratio of their potencies compared with DE particles in each of the four bioassays. For example, the epidemiology-based relative risk of exposure to 1 $\mu g/m^3$ of coke oven emissions was estimated to equal 4.4×10^{-4}. 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 assumed to equal 0.06 (slightly less than that in the Albert et al., 1983, studies). The diesel potency estimate using this comparison is then equal to $(0.53/2.1) \times 0.06 \times 4.4 \times 10^{-4}/\mu g/m^3$, or $6.6 \times 10^{-6}/\mu g/m^3$ DPM. A total of eight comparisons were made for each engine, four bioassays times two epidemiology-based potency estimates.

The Harris (1983) estimates are not comparable to those of Albert et al. (1983) without adjustment. The unit risk estimates of Albert and co-workers are based on absolute risk during lifetime exposure, whereas Harris reported his values in terms of relative risk per year of exposure. To adjust this to lifetime risk for continuous exposure, it is necessary to multiply

Harris' values by a factor of 2.7 = (70 × 0.039), where 70 reflects the lifetime exposure (70 years) and 0.039 is the lifetime lung cancer mortality rate in the U.S. population.

The range of potencies varied from 0.2×10^{-5} to 0.6×10^{-5} for the Nissan sample, 0.1×10^{-5} to 2.4×10^{-5} for the Oldsmobile 350, 0.2×10^{-5} to 27.8×10^{-5} for the Volkswagen Rabbit, and 0.1×10^{-5} to $2.5 \times 10^{-5}/\mu g/m^3$ DPM for the Caterpillar sample. Harris (1983) derived an overall mean relative risk value of $3.5 \times 10^{-5}/\mu g/m^3$ for the three light-duty engines with a 95% upper confidence limit of 2.5×10^{-4}. Individual mean values for each engine were not reported. After multiplying by 2.7 to convert to a unit risk, the upper-bound estimate of potency from the three light-duty engines was equal to $6.8 \times 10^{-4}/\mu g/m^3$ DPM. McClellan (1986), Cuddihy et al. (1981, 1984), and Cuddihy and McClellan (1983) estimated a risk of about $7.0 \times 10^{-5}/\mu g/m^3$ DPM using a comparative potency method similar to those reported in the preceding paragraph. The database was similar to that used by Albert et al. (1983) and Harris (1983). This estimate agrees quite well those reported by Albert et al. (1983). Although the Harris (1983) estimate is somewhat greater, it should be remembered that it was based on preliminary data.

With the availability of chronic cancer bioassays, more recent assessments were based on lung tumor induction in rats. Albert and Chen (1986) reported a risk estimate based on the chronic rat bioassay conducted by Mauderly et al. (1987). Using a multistage model and assuming equivalent deposition efficiency in humans and rats, they derived a 95% upper confidence limit of 1.6×10^{-5} for lifetime risk of exposure to 1 $\mu g/m^3$. Pott and Heinrich (1987) used a linear extrapolation, including data reported by Brightwell et al. (1989), Heinrich et al. (1986a), and Mauderly et al. (1987). They reported risk estimates of 6×10^{-5} to $12 \times 10^{-5}/\mu g/m^3$. More recently, Smith and Stayner (1990), using time-to-tumor models based on the data of Mauderly et al. (1987), derived 95% upper confidence limits ranging from 1.5×10^{-5} to $3 \times 10^{-5}/\mu g/m^3$. Pepelko and Chen (1993) developed unit risk estimates based on the data of Brightwell et al. (1989), Ishinishi et al. (1986), and Mauderly et al. (1987) using a detailed dosimetry model to extrapolate dose to humans and a linearized multistage model. Taking the geometric mean of individual estimates from the three bioassays, they derived unit risk estimates of $1.4 \times 10^{-5}/\mu g/m^3$ when dose was based on carbon particulate matter per unit lung surface area rather than whole DPM and $1.2 \times 10^{-4}/\mu g/m^3$ when based on lung burden per unit body weight. Hattis and Silver (1994) derived a maximum likelihood estimate for occupational exposure of $5.2 \times 10^{-5}/\mu g/m^3$ based on lung burden and bioassay data reported by Mauderly et al. (1987) and use of a five-stage Armitage-Doll low-dose extrapolation model.

Lung cancer risk estimates based on epidemiologic data were derived by two investigators. Harris (1983) 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: bus garage engineers, bus drivers, bus

conductors, engineers in central works, and motormen and guards. The first group was considered to have received the highest exposure; the next two, intermediate; and the last two, none. When cancer death rates for the high-exposure group were compared with those of London males, there was no increase in the observed-to-expected (O/E) ratios. The author, in fact, considered the results to be negative. However, because the low rate of lung cancer in all the LTA exposure groups may have been the result of a "healthy worker" effect, Harris (1983) compared the exposed groups with internal controls. He 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 garage engineers compared with bus drivers and conductors. Using this method, the relative risk of the exposed groups was greater than 1 but was statistically significant only for garage engineers exposed from 1950 to 1960. In this case, the O/E ratio was 29% greater than the presumed unexposed controls.

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 the cancer incidence; and
- Uncertainty in the mathematical specification of the model.

Taking the uncertainties into account, he derived a maximum likelihood relative risk estimate of 1.23×10^{-4} with a 95% upper confidence limit of $5 \times 10^{-4}/\mu g/m^3$ DPM per year. These estimates are equal to 5×10^{-4} and 2×10^{-3}, respectively, when converted to an absolute risk for lifetime exposure to 1 $\mu g/m^3$ particulate matter. It should be noted that, because of the high degree of uncertainty, the 95% lower confidence limit would predict no risk.

More recently, McClellan et al. (1989) reported risk estimates based on the Garshick et al. (1987) case-control study in which lung cancer in railroad workers was evaluated. Using a logistic regression, the expected relative risk of lung cancer death was estimated to rise 0.016 per year of exposure to DE. Adjustments were made to convert to continuous exposure (168 vs. 40 hours) for 70 years. Because exposure levels could not be defined exactly, two sets of calculations were made, assuming inhaled DPM concentrations of either 500 or 125 $\mu g/m^3$ DPM. Using a 95% upper confidence limit, the number of excess cancer deaths per year in the United States was estimated to range from 1,900 to 7,400. These values could then be converted to a lifetime 95% upper confidence limit of the risk of exposure to 1 $\mu g/m^3$ DE, by dividing the estimated excess number of annual cancer deaths in the United States by the total population and

multiplying by 70, the estimated mean lifespan. Using this approach, unit risks of 0.6×10^{-3} to 2×10^{-3} were derived. Even using the 95% lower confidence limits, an excess of 100 to 400 deaths is predicted, unlike the Harris (1983) study in which no excess deaths could be predicted based on the lower confidence limit. The estimates discussed in this section are listed in Table 11-1.

Each of the methods used to assess cancer risk has differing strengths and weaknesses, which will be discussed later in this chapter.

Table 11-1. Estimated 95% upper confidence limits of the lifetime risk of cancer from inhalation of 1 μg/m³ diesel particulate matter (DPM)

Method	Potency	Comments	Reference
Comparative potency	3.5×10^{-5}	Nissan engine	Albert et al., 1983
Comparative potency	2.6×10^{-5}	Average of 3 engines	Albert et al., 1983
Comparative potency	7.0×10^{-5}	Light-duty engines	Cuddihy et al., 1984
Comparative potency	6.8×10^{-4}	Average of 3 engines	Harris, 1983
Multistage model	1.6×10^{-5}	Lung cancer rats[a]	Albert and Chen, 1986
Straight-line extrapolation	$6\text{-}12 \times 10^{-5}$	Lung cancer rats[b]	Pott and Heinrich, 1987
Time-to-tumor model	$2\text{-}3 \times 10^{-5}$	Lung cancer rats[a]	Smith and Stayner, 1990
Logistic regression	8×10^{-5}	Lung cancer rats[c]	McClellan et al., 1989
Multistage model	1.4×10^{-5}	Lung cancer rats[d]	Pepelko and Chen, 1993
Armitage-Doll model	5.2×10^{-5}	Lung cancer rats[a,e]	Hattis and Silver, 1994
Epidemiologic analysis	1.4×10^{-3}	London transport study	Harris, 1983
Epidemiologic analysis	$.6\text{-}2 \times 10^{-3}$	Railroad workers	McClellan et al., 1989

[a]Used data from studies by Mauderly et al., 1987.
[b]Used data from studies by Brightwell et al., 1989; Heinrich et al., 1986a; and Mauderly et al., 1987.
[c]Used data from studies by Brightwell et al., 1989; Ishinishi et al., 1986; Iwai et al., 1986; and Mauderly et al., 1987.
[d]Used data from studies by Brightwell et al., 1989; Ishinishi et al., 1986; and Mauderly et al., 1987.
[e]Maximum likelihood estimate based on 53 years of exposure, 8 hours/day, 240 days/year.

11.3.2. Current Approaches to Quantitation of Human Cancer Risk From Exposure to Diesel Exhaust

11.3.2.1. *Dose-Response Estimates Using Human Epidemiologic Data*

In an attempt to quantitatively estimate risk using humans, a detailed analysis of the Garshick et al. (1988) study of railroad workers was carried out by ICF Clements (Crump et al., 1991). This study was selected because the cohort was quite large, significant increases in relative risk ratios were reported, exposure data collected during the later years of exposure were available, and some estimates of early exposures were available (Woskie et al., 1988a,b). The investigators were also able to eliminate many of the confounding factors present in earlier studies. Garshick et al. (1988) analyzed information obtained from the Railroad Retirement Board on 55,407 white males who began railroad employment between 1939 and 1949, who were between the ages of 40 and 64 in 1959, and who in 1959 worked at 1 of the 39 jobs selected to represent a range of potential DE exposure. Two analyses that indicated effects of exposure to DE on lung cancer risk in this cohort were reported: (1) A relative risk for lung cancer of 1.45 (95% confidence interval [CI] = 1.11, 1.89) was observed for DE-exposed workers who were 40 to 44 years of age in 1945 and who consequently had the longest potential exposure to DE; relative risk was progressively lower among DE-exposed workers who were older in 1959 and who had potentially shorter exposures to DE; and (2) the relative risk of lung cancer increased monotonically with increasing duration of work in 1959 or later in a job involving DE exposure (disregarding exposures in the current year and in the most recent 4 years); this risk was 1.72 (95% CI = 1.29, 2.23) in the group with the longest exposure (15 to 17 years).

The Garshick et al. (1988) database was used by two groups of investigators for the purpose of quantitating cancer risk from exposure to diesel engine emissions (Crump et al., 1991, unpublished; Dawson, California EPA, personal communication to William Pepelko, U.S. EPA). The results of these analyses were not in agreement. While Crump and co-workers failed to derive a positive dose-response relationship, Dawson did report increasing incidence of lung cancer with exposure level. These differences have not been resolved. Until they are resolved, utilization of the Garshick et al. (1988) study to quantitate cancer risk from DE is not anticipated. It should be noted, however, that despite the decision to not use this study for quantitative analysis at this time, it still provides the strongest qualitative evidence for a causal association between DE exposure and lung cancer.

As described previously, McClellan et al. (1989) estimated annual lung cancer mortality in the U.S. population, per $\mu g/m^3$ DPM, using the Garshick et al. (1987) case-control study, which minimizes the issue of the dose response by assuming a single exposure level for all the cases. Mean exposure levels of 125 or 500 $\mu g/m^3$ DPM were used for deriving risk estimates because exposure data, while limited, indicated that actual exposures were unlikely to fall outside

this range (Woskie et al., 1988a,b). The unit risk estimate of $2 \times 10^{-3}/(\mu g/m^3)$, assuming a mean exposure concentration of 125 $\mu g/m^3$, is then quite conservative because it is based not only on the upper-bound estimate of lung cancer mortality, but also on the lower estimate of mean exposure. The risk estimates of either 0.6 or $3 \times 10^{-3}/(\mu g/m^3)$, depending on the level of exposure assumed, will be approximately halved if maximum likelihood estimates of mortality are used.

11.3.2.2. *Dose-Response Estimates Based on Animal Bioassay Data*

Several cancer potency estimates for DE have been developed by U.S. EPA and others. Most of them used somewhat similar approaches, and as a result most of the estimates did not differ greatly. The approaches used here are an extension of these efforts. To develop unit risk estimates, it was necessary to address several issues, including (a) determination of the critical target site for DE; (b) determination of the fraction of exhaust responsible for tumor induction; (c) use of existing methods or development of new dosimetric methods for accurately extrapolating dose from experimental animals chronically exposed to high concentrations of exhaust to humans exposed at ambient concentrations; and (d) selection of the most suitable low-dose risk extrapolation model.

The critical target organ was considered to be the lung. Although Iwai et al. (1986) reported the induction of malignant lymphomas in the spleen as well as lung tumors in rats following DE exposure, the lung was the only target site in other experimental studies with this species. Potential carcinogenic agents present in DE may be adsorbed from the lungs, enter the bloodstream, and be transported systemically. Although increases in DNA adduct levels in peripheral lymphocytes have been reported in workers occupationally exposed to DE (Hemminki et al., 1994), there is insufficient evidence for induction of cancer in humans at systemic locations. Particle-adsorbed organics also may reach systemic targets via the gastrointestinal tract. Particles deposited in the conducting airways are cleared to the mouth quite rapidly and swallowed. A considerable volume of particles are also likely to be ingested as a result of grooming during whole animal exposures (Wolff et al., 1982), resulting in the possible uptake of carcinogens by the gastrointestinal tract. Because half-times for elution of organics from the particles are considerably longer than passage through the gastrointestinal tract, however, the fraction adsorbed is expected to be small.

The site of action in the lungs is assumed to be the epithelial lining of the alveoli and small airways. According to Mauderly et al. (1987) and others, inflammation and tumors appear to arise from this tissue. Although a connection between interstitial events and lung tumors has been suggested for particles (i.e., fibrosis as a precondition for lung tumors [Kuschner, 1984]), data are unavailable to support this view with respect to DE-induced tumors.

Accurate extrapolation of dose from experimental studies using animals exposed at high concentrations of exhaust to humans exposed to ambient concentrations requires a variety of adjustments. These include adjustments for species differences in deposition efficiency and respiratory exchange rates. An important factor is the rate of particle clearance from the deep lung. Normal clearance half-times from the alveolar region are several times longer in humans than in rats (Chan et al., 1981; Bohning et al., 1982; Griffis et al., 1983). This may result in an underestimate of lung burden when extrapolating to humans. On the other hand, the high exposure concentrations used in some of the animal studies resulted in greatly slowed or even complete cessation of macrophage-based clearance (Chan et al., 1984).

To more accurately extrapolate dose from experimental studies to humans, detailed dosimetry modeling was used to account for these factors. One of these models (Yu et al., 1991) is listed in Appendix C. A second approach reported by Hattis and Silver (1994) was also employed for comparison. Hattis and Silver determined area under the curve/time, using observed lung burden data reported by Mauderly et al. (1987). Human lung burden in the Hattis and Silver approach was calculated using a one-compartment model suggested by Smith (1992), assuming that at low doses the lung burden is at steady state. Dose for both approaches was estimated in terms of either particle concentration per unit of lung surface area or lung burden/body surface area.

The use of whole particle lung burden as the dosimeter is based on the assumption that both the diesel particle as well as its surface-adsorbed constituents are responsible for the carcinogenic effects of DE. A particle-based assessment was considered to be reasonable for two principal reasons. First, exposure to the vapor phase alone did not result in detectable tumor induction in rats (Ishinishi et al., 1986; Iwai et al., 1986; Stöber, 1986; Brightwell et al., 1989; Heinrich et al., 1995). Second, while tumor induction at high doses is considered to be primarily a particle effect (Heinrich, 1990; Heinrich et al., 1995; Nikula et al., 1995), the particle-associated organics are likely to play a greater role at lower doses. Particle concentration is also the most practical measure of dose because of the large number of surface-associated compounds and because their concentrations are seldom reported.

As reviewed in Chapter 7, several bioassay studies showed positive lung tumor responses in rats (Ishinishi et al., 1986; Iwai et al., 1986; Stöber, 1986; Mauderly et al., 1987; Brightwell et al., 1989; Heinrich et al., 1995; Nikula et al., 1995). Three of these studies (Tables 11-2 through 11-4) were used for unit risk calculations because they were designed using multiple exposure groups and thus are more appropriate for risk calculations. Use of these studies also allows comparison with earlier risk estimates. The time-to-event (i.e., death with or without tumors) data are available for the Mauderly et al. (1987) study. These time-to-event data are used in all

Table 11-2. Incidence of lung tumors in Fischer 344 rats (males and females combined) exposed to heavy-duty engine exhaust

Exposure concentration (mg/m^3)	Dose metric: Weekly exposure ($mg/m^3 \times h$)[a]	Dose metric: Lung particle burden (mg/cm^2 lung surface)[b]	Lung tumor incidence[c]
0	0	0	2/230
0.35	12	6.4×10^{-5}	3/223
3.50	122	2.8×10^{-3}	6/222
7.08	248	6.0×10^{-3}	18/227

[a]Exposures were 7 hours/day, 5 days/week for 30 months. Numbers include those dying or moribund prior to 30 months.
[b]Calculated using mathematical models in Appendix C.
[c]Does not include squamous cysts.

Source: Mauderly et al., 1987.

Table 11-3. Incidence of lung tumors in Fischer 344 rats (males and females combined) exposed to heavy-duty engine exhaust

Exposure concentration (mg/m^3)	Dose metric: Weekly exposure ($mg/m^3 \times h$)[a]	Dose metric: Lung particle burden (mg/cm^2 lung surface)[b]	Lung tumor incidence[c]
0	0	0	1/123
0.46	44	2.5×10^{-4}	1/123
0.96	92	2.0×10^{-3}	0/125
1.84	177	4.2×10^{-3}	4/123
3.72	357	8.8×10^{-3}	8/124

[a]Exposures were 16 hours/day, 6 days/week for 30 months. Numbers include those dying or sacrificed moribund before 30 months.
[b]Calculated using mathematical models in Appendix C.
[c]All tumors reported were carcinomas.

Source: Ishinishi et al., 1986.

Table 11-4. Incidence of lung tumors in Fischer 344 rats (males and females combined) exposed to diesel engine exhaust

Exposure concentration (mg/m^3)	Dose metric: Weekly exposure ($mg/m^3 \times h$)[a]	Dose metric: Lung particle burden (mg/cm^2 lung surface)[b]	Lung tumor incidence[c]
0	0	0	4/250
0.7	56	3.5×10^{-4}	1/112
2.2	176	4.2×10^{-3}	14/112
6.6	248	1.3×10^{-2}	55/111

[a]Exposures were 16 hours/day, 5 days/week.
[b]Calculated using mathematical models listed in Appendix C.
[c]The number of animals sacrificed at 6 and 12 months is excluded from the denominators.

Source: Brightwell et al., 1989.

the risk calculations based on the Mauderly et al. (1987) data. Use of a time-to-tumor model, when data are available, allows more accurate modeling of dose response.

Risk estimates were derived using two models. The first one is the linearized low-dose extrapolation model. The linearized multistage (LMS) model is adopted by U.S. EPA as a default procedure to provide an upper bound estimate of risk when data useful to incorporate plausible mechanisms are not available. This model was selected as one of the approaches because, although mechanisms of carcinogenesis have been proposed (see Chapter 10), they remain largely unproven.

The LMS model has the mathematical form $P = 1 - \exp(-Z)$, where Z is either $Z = q_0 + q_1xd + ... + q_mxd^m$, a polynomial of concentration d, or $Z = (Q_0 + Q_1xd + ... + Q_mxd^m)\, xt^k$, a polynomial of concentration d multiplied by a time factor t^k when time-to-event data are used. The range of extrapolation is about three orders of magnitude in the present study. Because the extra risk $(P - P_0)/(1 - P_0)$ is dominated by the linear term q_1xd at low concentration, the 95% upper bound of q_1 is used to represent unit risk when d is expressed in $\mu g/m^3$. P_0 denotes the lifetime cancer risk at concentration 0. The resulting unit risk estimates are listed in Table 11-5.

Table 11-5. Unit risk estimates per μg/m³ of diesel exhaust based on the Yu et al. (1991) dosimetry model

	Lung burden/lung surface area	Lung burden/body weight$^{2/4}$
Mauderly et al., 1987	3.4×10^{-5}	9.9×10^{-5}
Ishinishi et al., 1986	1.6×10^{-5}	4.6×10^{-5}
Brightwell et al., 1989	7.1×10^{-5}	2.1×10^{-5}
Geometric mean of three studies	3.4×10^{-5}	9.9×10^{-5}

The potency estimates derived using the LMS model are an improvement over most of the earlier estimates because of improved methods for extrapolating dose from experimental animals to humans. These assessments as well as earlier ones, however, are based on the premise that carcinogenic effects are a function of the whole particle concentration. Moreover, risk estimates are calculated using the default assumption that responses vary linearly with particle concentration even at ambient exposure concentrations. As noted in Chapter 10, however, while particle effects may be dominant at large lung burdens, other components such as polycyclic aromatic compounds, nitroaromatics, and surface-associated reactive oxygen species are likely to play a more important role as exposure concentrations approach those found in the ambient air.

In an attempt to account for the effects of particles as well as particle-associated components of DE, a biologically based two-stage model with piecewise constant parameters was developed. This model assumes that both the carbon and the organic fraction have initiating properties and that the carbon fraction also has effects on the proliferation, conversion and progression steps of carcinogenesis. In addition, it accounts for the tumor initiating and promoting effects at high exposure concentrations. A description of this model has been published by Chen and Oberdörster (1996). (See Appendix B for details.) The resultant two-stage model is then used to predict tumor responses under various exposure scenarios and to investigate the impacts of different biological assumptions on risk projection.

The parameters for the two-stage model were statistically estimated initially, using tumor response data from animals exposed to high concentrations of DE and assuming particles continue to exert effects at low concentrations (i.e., nonthreshold for particle effects). When the two-stage biologically based dose-response model (BBDr) was compared with results derived with the LMS model, using malignant tumor data from the Mauderly et al. (1987) study, the results were nearly identical (Table 11-6). The maximum likelihood estimate (MLE) of cancer risk for the BBDr model at an exposure concentration of 1 μg/m³ was calculated to be 8.2×10^{-6}

Table 11-6. Relative importance of particles and organics at different exposure concentrations assuming either threshold or nonthreshold effects of particles

Excess risk in humans (MLE)			
Concentration	**Threshold effect of particles**	**Nonthreshold effect of particles**	**Ratio[a]**
0.1	1.8×10^{-7}	8.1×10^{-7}	4.5
1.0	1.8×10^{-6}	8.2×10^{-6}	4.6
100	1.2×10^{-2}	5.6×10^{-4}	4.9
1,000	1.9×10^{-3}	2.6×10^{-2}	14.1

[a]Ratio =ratio of column 3 and column 2.

with an upper 95% confidence limit of 1.7×10^{-5}. The 95% upper confidence limits using the LMS model were also 1.7×10^{-5} when rounded to two significant numbers.

Table 11-6 demonstrates that risk is much smaller if the assumption is made that particles do not exert effects below a certain concentration. As can be seen in Table 11-6, risk is decreased 4.6-fold at an exposure concentration of 1 $\mu g/m^3$ if a threshold is assumed for particles. Importantly, not only is risk decreased if a threshold is assumed for particle effects, but while particles play a major role in tumor induction at high exposure concentrations, organics and other components of DE play an increasingly dominant role as concentrations become lower.

An alternative approach that avoids the controversies related to lung overload with concomitant pathology involves calculation of an upper-bound risk based only on animals exposed to low diesel particle concentrations (e.g., below 0.5 mg/m^3). This modeling is designed to cap a theoretical upper-bound estimate on a nonobservable rat resonse to organics, while assuming organics and possibly free radicals are driving carcinogenicity at low exposure concentrations. As a general matter, the newly proposed Carcinogenicity Risk Assessment Guidelines suggest identifying a point of departure on the dose-response curve, perhaps an ED_{10} or some other ED_x, and then extrapolating risk from that point of departure. The low-dose groups from the Mauderly et al. (1987) and Ishinishi et al. (1986) studies are the most suitable for this purpose. Both include exposure groups at less than 0.5 mg/m^3, and have the same duration follow-up (30 mo). By combining studies, statistical power is increased, resulting in a smaller upper-bound estimate of risk than by either study alone. The resultant upper-bound estimate is equal to $1.9 \times 10^{-5}/\mu g/m^3$.

While this approach has the advantage of avoiding controversies related to lung particle overload, it also has disadvantages. First, because lung tumors were not significantly increased

at the doses used, the estimated risk is considered unlikely to exceed the upper bound, but may be as low as zero. Second, the upper-bound estimate of risk is greater than the unit risk estimates derived using data from animals exposed to all concentrations of DE.

11.3.2.3. *Dose-Response Estimation Using Benzo(a)pyrene as a Dosimeter*

Pike and Henderson (1981) found good agreement when relating the concentration of benzo(a)pyrene (B[a]P) to lung cancer risk in smokers, British gas workers, U.S. coke oven workers, and U.S. hot pitch workers and when comparing residents of rural and urban locations. They concluded that while B[a]P is unlikely to be the only carcinogen present and perhaps not even the most important one present in combustion emissions, nevertheless it serves as a reasonably accurate dosimeter. Based on an estimated cancer risk of 1/1,500 per ng/m^3 B[a]P and a reported B[a]P concentration of 3.9 ng/μg DPM in exhaust from a Volkswagen engine (Heinrich et al., 1995), a maximum likelihood estimate of cancer risk from lifetime exposure to 1 μg/m^3 can be calculated to be 3×10^{-6}. The 95% upper bound was not derived, but is estimated to be near 1×10^{-5}.

11.3.3. Strengths and Weaknesses of Differing Approaches for Quantitating Cancer Risk

11.3.3.1. *Dose-Response Estimates Based on Human Epidemiologic Data*

A major advantage in the use of human data is the elimination of uncertainty due to possible differences in sensitivity to cancer induction by DE among species. As will be discussed later, there is some evidence that rats may respond differently from humans to DE. Second, epidemiology studies are based on occupational exposures, which generally occur at concentrations insufficient to result in lung particle overload. Thus, lung cancer in the human studies is likely to be induced by non-particle-overload mechanisms under either occupational or ambient exposure levels. Cancer induction in the rat bioassays, on the other hand, was observed only under particle-overload conditions, with concomitant pathology. Uncertainty in extrapolating from occupational studies is therefore decreased, not only because low-dose extrapolation occurs over a smaller range, but because mechanisms of cancer induction are less likely to vary within this range with accompanying changes in the dose-response curve.

There is considerable evidence for nonoverload mechanisms of cancer induction by products of fossil fuel combustion. Mumford et al. (1989) reported greatly increased lung cancer mortality in Chinese communes burning so-called smoky coal containing high concentrations of PAHs. Demonstration of the carcinogenicity of coke oven emissions in humans (Lloyd, 1971) also provided evidence for a role by organics because coke oven PM contains a high concentration of PAHs but lacks an insoluble carbon core. Increased levels of aromatic DNA adducts were reported in bus maintenance and terminal workers by Hemminki et al. (1994) and

in garage workers and mechanics exposed to diesel exhaust (Nielson and Autrup, 1994). Studies by Sagai et al. (1993) have indicated that DPM could produce superoxide and hydroxyl radicals in vitro, without any biologically activating systems. On the basis of these findings, they suggested that most DE toxicity in lungs is due to active oxygen radicals. In a more recent study, these investigators reported that instillation of only 0.1 mg of DPM into mouse lungs resulted in the production of 8-hydroxyguanosine in lung cell DNA. The critical lesion may thus be induced by oxygen free-radicals generated from DPM (Nagashima et al., 1995).

An uncertainty associated with most of the diesel epidemiology studies was the inability to completely eliminate all confounding factors, resulting in possible errors in estimating relative risk ratios. Small errors in adjustment for smoking, for example, can result in considerable error because smoking has a much larger effect on relative cancer risk than is likely for DE. The likelihood of significant confounding errors in the Garshick et al. (1987 and 1988) studies is decreased by the considerable effort exerted to eliminate or reduce such factors, especially smoking. Moreover, a meta-analysis by the California EPA (Cal-EPA, 1997) and more recently one by Bhatia et al. (1998) using a number of diesel epidemiology studies resulted in relative risk ratios quite similar to the one reported by Garshick et al. (1987). Although exposure levels are likely to have differed somewhat among studies, the agreement still suggests that a relative risk near 1.4 is reasonable.

The greatest uncertainty in estimating DE-induced cancer risk from epidemiology studies is determination of exposure levels. Even though DPM concentrations were often measured near the end of the studies, historic exposure data are generally lacking. Such information is critical, since there is indirect evidence, based on other pollutant measurements such as nitrogen oxides, that exposure levels have decreased considerably in recent years, especially in the railroad industry (Woskie et al., 1988b). In the only historic study found in which DPM were measured, Heino et al. (1978) reported average concentrations of 2 mg/m^3 in Finnish roundhouses. Woskie et al. (1988a), by contrast, reported a mean of 134 $\mu g/m^3$ for roundhouse workers near the end of the Garshick et al. (1987, 1988) studies. While the relationship between DPM concentrations in Finnish and U.S. railroad roundhouses during the 1970s is uncertain, it does point to the likelihood that exposure levels have decreased over time.

Despite the uncertainties discussed above, the epidemiology data-based risk assessments are useful for bounding risk. The control of confounding factors in the Garshick et al. (1987) study and the general agreement of relative risk estimates with those of other studies renders it unlikely that the true risk is greatly different from the reported one. The mean DPM concentration for all the occupational groups was somewhat less than 125 $\mu g/m^3$. Historic exposures were undoubtedly greater, but unlikely to approach 2 mg/m^3 because the cases in this study included not only roundhouse workers but other occupational groups as well, who were

likely exposed at lower concentrations. Based on the evidence available, there is considerable confidence that mean exposures are in the range of 125-500 $\mu g/m^3$.

11.3.3.2. *Dose-Response Estimate-Based Laboratory Animal Bioassays*

Cancer risk assessment from exposure to DE, based on available animal bioassays, has a variety of strengths. The studies selected are adequately designed, eliminating confounding factors often present in epidemiology studies. Exposure duration and exposure levels were precisely controlled and monitored. The presence or absence of tumors were verified by pathological evaluation. Although animal-to-human extrapolation of dose is required, the development of dosimetry models has eliminated much of the uncertainty in this area.

Nevertheless, two important uncertainties remain: the adequacy of the rat as a model for evaluating human risk of cancer from exposure to DE and the shape of the dose-response curve. It is believed by some that the rat may be unique in its response to particulate matter, and therefore its use for assessing human lung cancer risk is questionable (Mauderly, 1994; Watson and Valberg, 1995). As noted in Chapter 7, the rat is the only species that has unequivocally been shown to develop lung cancer in response to DE exposure. On the other hand, only two other species, Syrian hamsters and mice, have been tested adequately. Although responses in the hamster have been negative, this species is known to be resistant to induction of lung cancer (Heinrich, 1994). Mice also fail to respond in standard bioassays of DE (Heinrich et al., 1995; Mauderly et al., 1996). Positive results, however, have been reported in mice exposed from birth or conception (Pepelko and Peirano, 1983; Takemoto et al., 1986), a condition likely to increase sensitivity. In the case of the study reported by Pepelko and Peirano, Sencar mice were used, a strain sensitive to chemical induction of skin tumors. Since the alveolar lining of the lung is also epithelial tissue, it is possible these mice are more sensitive to the organic constituents of DPM. Ichinose et al. (1997a,b) reported increased lung tumors in mice following intratracheal instillation of DPM. Positive results in mice may have therefore been due to use of a sensitive strain, early exposure to increased sensitivity, or very high local concentrations due to the instillation procedure.

It has also been argued that humans are resistant to particle-induced lung cancer; although coal miners develop pneumoconiosis, lung cancer seldom occurs. Rats, on the other hand, were reported to develop lung cancer in response to coal dust (Martin et al., 1977). This study, however, was poorly described, and the number of animals exposed was small (4/36 developed lung cancer). Moreover, exposure levels were very high and lung burdens were greater than those generally encountered in coal miners (Mauderly, 1994). Finally, although lung cancer has not been reported in most epidemiology studies of coal miners, Zhong and Dehong (1995)

reported that Chinese workers suffering coal miners' pneumoconiosis did have an increased risk of lung cancer.

The evidence for the uniqueness of the rat carcinogenic response to DE is therefore still equivocal. Nevertheless, the evidence to date does raise questions concerning the suitability of the rat for assessing human risk from exposure to DE. This concern is heightened by the fact that particle deposition patterns are different in the rat and human. Because of the absence of respiratory bronchioles in the rat, a greater fraction of inhaled particles deposit in the alveolar regions; in primates, deposition occurs to a large extent at the bifurcation of the small bronchi. Differing deposition patterns may result in different pathologic responses, as reported by Nikula et al. (1997) for rats and monkeys.

The other important uncertainty relating to use of rat bioassay data concerns low-dose extrapolation. Significant lung tumor increases in experimental animals have generally been obtained only at concentrations resulting in lung particle overload with concomitant pathological effects. A hypothesis first proposed by Vostal et al. (1986) is based on the belief that diesel particles themselves induce lung cancer, through a secondary effect. Driscoll (1995) suggested that the secondary effect was due to release of various inflammatory mediators by particle overloaded phagocytic cells. The resultant inflammatory response, with accompanying cell division, can increase the likelihood that any oxidant-induced or spontaneously occurring genetic damage becomes fixed in a dividing cell and is clonally expanded. In support of this hypothesis are the observations that lung cancer can be induced by inhalation of so-called "inert" particles, such as titanium dioxide (Lee et al., 1986) and coal dust (Martin et al., 1977), or by intratracheal instillation of activated carbon (Kawabata et al., 1986). Inhalation of carbon black that was virtually devoid of organics and is similar to the carbon core of diesel exhaust was also reported to induce lung cancer in rats (Heinrich, 1990; Heinrich et al., 1995; Nikula et al., 1995). Finally, Kawabata et al. (1993) induced lung tumors in rats through intratracheal instillation with DPM from which the organic components had been extracted.

Although considerable evidence for the particle-overload hypothesis exists, occupational exposures in which lung cancer induction has been reported in humans are generally considered insufficient to induce lung particle overload. This suggests that other mechanisms are also likely to be operating. Experimental evidence also provides support for low-dose mechanisms. Riebe-Imre et al. (1994) reported that carbon black is taken up by lung epithelial cells in vitro, inducing chromosomal damage and disruption of the cytoskeleton (lesions that closely resemble those in tumor cells) at concentrations that did not induce measurable toxicity. As noted in the previous section, diesel particles are capable of inducing toxicity and possible carcinogencity at low doses through production of oxygen free radicals. Finally, Dasenbrock et al. (1996) reported that extraction of the organic fraction from diesel particles decreased their carcinogenic potency, even

though specific particle surface area, a factor related to cancer potency, is increased following extraction.

In summary, the use of rat data for quantitating human lung cancer risk from exposure to DE results in considerable uncertainty. There is some doubt concerning the validity of the rat model for this purpose, although proof is still lacking. The greatest uncertainty concerns low-dose extrapolation of lung cancer risk. Information to date indicates that DE induces lung cancer by more than one means, including particle overload-induced pathology, carcinogenic organics present on the particle, and oxygen free radicals. While all these methods are likely to function at high doses, particle effects are likely to be absent or minimal at low exposures, resulting in a high degree of uncertainty regarding the shape of the dose-response curve.

Despite these uncertainties, rat data are still useful for comparison with other estimates, especially those derived using human epidemiology data. The greater risk estimates derived using epidemiology data, for example, suggest that even though rats are the only laboratory species in which lung cancer can be regularly induced by DE and other fine particulate matter, they may be less sensitive than humans to nonoverload mechanisms induced by DE at low concentrations. Unfortunately, limitations on animal numbers do not allow testing this possibility.

11.3.3.3. *Dose-Response Estimates Based on the Biomarker Approach*

This approach provides reasonably good estimates of lung cancer risk in spite of the fact that B(*a*)P may constitute a relatively small fraction of the carcinogens present in combustion and pyrolysis products of coke ovens, hot pitch gas productions, etc. Risk estimates were also based on well-documented lung cancer rates in the occupationally exposed groups. On the other hand, while predictions are good for the pollutants tested, the particles present, unlike diesel particles, generally lack an insoluble carbon core. As noted below, adsorption to an insoluble particle core may influence risk estimates. Estimates of cancer risk will also vary based on B(*a*)P concentration on the particle. The variability in B(*a*)P concentration among different DE sources and its effect on cancer potency have not been evaluated.

11.3.3.4. *Dose-Response Estimates Based on the Comparative Potency Approach*

In this method, the potency of diesel DPM extract is compared with other combustion or pyrolysis products, coke oven emissions, roofing tar, and cigarette smoke condensate for which epidemiology-based unit risk estimates have been developed. Comparisons are made using short-term tests such as skin painting, mutation, and mammalian cell transformation. The ratio of the potency of DPM extracts to each of these agents is then multiplied by their unit risk estimates to obtain the unit risk for DE. Because no new studies have been carried out since the

early 1980s, the values considered are those published by Albert et al. (1983) and listed in Table 11-1.

This test is based on the belief that cancer induction at low doses is due to the organic fraction present on diesel particles. A major strength of this approach is avoidance of lung particle overload effects. The tests also have shown that the organic fraction of DE can induce cancer. A degree of uncertainty is due to the possibility that the presence of particles may alter the carcinogenicity of the organics. For example, particle-associated organics may deposit in different regions of the lungs than free organics do. Association with particles may increase residence time, thus increasing potency. On the other hand, a fraction of the organics may not be bioavailable because of tight adherence to the particle. Another uncertainty involves the assumption that cancer potency in short-term tests is the same as that for lung cancer induction. This is somewhat mitigated by the fact that the assessment is based to a large degree on skin painting studies. Skin and lung surfaces are both epithelial tissue, increasing the likelihood that responses will be similar. Nesnow et al. (1995) have, in fact, showed that for many polycyclic aromatic compounds relative cancer potency by skin painting and lung adenoma induction in strain A mice was quite similar.

11.4. SUMMARY AND RECOMMENDATIONS

Based on limited evidence from human epidemiologic studies and adequate evidence from animal experiments, DE is considered to be a probable human carcinogen. The evidence places DE in the upper range of agents classified as probable human carcinogens because of positive findings in both animal experiments and human data as well as the presence of known carcinogens in the mixture. The inability to completely eliminate confounding factors in most of the epidemiology studies, the lack of clearcut carcinogenic responses in more than one animal species, and the low concentration of organics precluded classifying DE as a known human carcinogen.

Cancer potency has been estimated using human epidemiology data, chronic animal cancer bioassays, a comparative potency approach, and use of B[a]P as a dosimeter. Each of these methods has important uncertainties that preclude recommendation of a point estimate of risk. Uncertainties relating to use of epidemiology studies include limited historical exposure data, inability to eliminate all possible confounding factors, and small increases in relative risk that can be more easily influenced by confounding factors. Uncertainties in use of chronic animal bioassays stem from questions regarding relative sensitivities of rats and humans to DE and shape of the low-dose response curve. In the comparative potency method, the assumption that relative potency in short-term tests accurately reflects relative cancer potency of different combustion emissions requires confirmation. While use of B[a]P as a dosimeter has been shown

to reflect the potency of several combustion emissions reasonably well, DE differs from these emissions because of the presence of an insoluble carbon core.

Despite these uncertainties, they are considered, collectively, adequate to place reasonable bounds on risk. An upper-bound lung cancer risk from lifetime exposure to DE at a particulate matter concentration of 1 $\mu g/m^3$ of 2×10^{-3}, based on the estimate derived by McClellan et al. (1989), using the case-control study of Garshick et al. (1987) and assuming a mean exposure concentration of 125 $\mu g/m^3$ is recommended. It is considered to be a reasonable upper bound because relative risk ratios were either less or only slightly greater in other epidemiology studies with DE. Furthermore, exposures were unlikely average less than 125 $\mu g/m^3$ particulate matter assumed. A lower bound of $1 \times 10^{-5}/(\mu g/m^3)$ is recommended. MLEs slightly less than this value were obtained from animal bioassay data by Chen and Oberdörster (1996) using a biologically based dose-response model and assuming nonthreshold effects for particles and by estimates based on use of B[a]P as a dosimeter according to the Pike and Henderson (1981) report and B[a]P content of DE reported by Heinrich et al. (1995). Most comparative potency estimates were only slightly greater than $1 \times 10^{-5}/\mu g/m^3$. The lower bound is considered to be reasonable for at least two reasons. First, the latter two approaches are based on effects of the organic constituents. Second, while they are likely to be the primary cause of lung cancer at low doses, other factors such as particle effects and reactive oxygen species may play some role. Thus, risk is unlikely to be significantly lower. This conclusion is supported by animal data-based calculations of Chen and Oberdörster (1996) using a biologically based, low-dose extrapolation model.

Finally, the 95% lower-bound estimate of risk, assuming exposures of 500 $\mu g/m^3$, is $5 \times 10^{-5}/\mu g/m^3$. The range of human data-based risk estimates therefore encompasses a large portion of the recommended bounds of 1 to $200 \times 10^{-5}/\mu g/m^3$.

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12. HEALTH RISK CHARACTERIZATION FOR DIESEL ENGINE EMISSIONS

12.1. INTRODUCTION

12.1.1. Scope

Earlier chapters focused on specific risk assessment topics and developed key findings for these topics or provided an overview of relevant background information. This chapter will integrate and summarize the key findings about the health hazards and risk potential for humans exposed to diesel exhaust (DE). This chapter will first integrate the key information and pertinent uncertainties for two of the three basic components of risk assessment: hazard identification and dose-response assessment. Exposure assessment, the other basic component, is not in the scope of this report, though an exposure perspective is included as background information. The final section will characterize the hazards and risk in a plain-language summary form.

For introductory purposes, a quick overview of findings in the key assessment areas will help put the remainder of this chapter into perspective.

- The DE particle and its coating of organics, as well as the accompanying gases and semivolatiles, have biochemical and toxicological properties that raise a suspicion about adverse health effects given sufficient exposure. Because DE is found only as a mixture, the choice of dosimeter for measuring exposure is an important issue. For DE, $\mu g/m^3$ of diesel particulate matter is used.
- Carcinogenicity: Epidemiologic data are strongly suggestive of a carcinogenic hazard to the lung under occupational exposure conditions. Some rat and mouse studies show a similar effect at high test exposures. Mode-of-action information poses the challenge of sorting among high-dose particle effects and possible low-dose effects from mutagenic/genotoxic organics.
- Noncancer toxicity: For chronic exposure, there is limited human and much animal evidence for adverse respiratory effects, such as airway restriction, other measures of reduced pulmonary function, and immunologic allergenic reactions. Acute exposure in humans elicits various reactions from some individuals, ranging from annoying or temporarily debilitating symptoms reflecting tissue irritation, up to permanent harm to the respiratory system from very high acute exposure episodes.
- Ambient exposures to DE vary widely depending on whether an occupational element is involved, the setting is urban or rural, or near to or distant from a source of DE emissions. General average ambient exposures run in the 0.6 - 3.2 $\mu g/m^3$ (of particulate) range, though some areas can be in the 4-22 $\mu g/m^3$ range at certain periods during the year.

12.1.2. What Is Diesel Exhaust in a Risk Assessment Context?

As reviewed in more detail in Chapter 2 and other chapters, a health risk characterization for DE is more complicated than for most environmental pollutants because DE is a complex mixture. The mixture consists of particulate matter made up of an elemental carbon core with

hundreds of compounds adsorbed to the surface (particle-phase emissions) and a volatile fraction that is also made up of many organic and inorganic compounds (gaseous-phase emissions). The particles, the particle coating of adsorbed compounds, and the volatile elements each have known properties from which hazards can be inferred, in addition to the aggregate hazard potential posed by the whole mixture.

The exhaust particles are formed through the condensation of even smaller particles in the engine and engine exhaust pathway. They average about 0.2 μm in diameter and have a very large surface area (50-200 m^2/gm). The main constituent is carbon, which accounts for approximately 80% of total particle mass. Approximately 70% of the total carbon occurs in the form of elemental carbon, the remainder being various organic compounds, called organic carbon. The particle constituents coating the particle surface include inorganics and hundreds of hydrocarbons. At least 16 hydrocarbons adsorbed onto the particles have been classified as having a carcinogenic potential for humans. Many of the compounds emitted as gases are potentially carcinogenic or otherwise toxic at some dose. These include benzene, 1,3-butadiene, various aldehydes, ethylene dibromide, nitroaromatics, oxides of nitrogen, and sulfur compounds. Additionally, there is evidence that reactive oxygen or hydroxal species (free radicals) may be formed on the particle surface that could cause or exacerbate damage to biological cells. Inorganic compounds are also present, including nitrates and compounds of sulfur.

The quantitative physical-chemical composition of DE exhaust is variable and depends on numerous factors, including operating conditions, heavy-duty versus light-duty engines, engine design, engine age, fuel technology, and exhaust control technology. Heavy-duty and off-road diesel engines have the largest U.S. particulate emissions. The human and animal health studies are pegged to specific engine exhaust generated at some time in the past, so the question of how relevant those exposures are to current conditions is a valid inquiry. There is no single answer to this question. However, health studies focus on particle mass as a surrogate for the DE mixture, so as the mass changes so may the applicability of the assessment findings.

Once diesel emissions are released in the air, they are subject to dispersal, dilution, and chemical and physical transformations. Newly emitted exhaust is "fresh" and has free radicals that pose some extra hazard, the magnitude of which is not discernable. DE that is more than a day old is "aged," largely because of atmospheric alterations, and is thought to have fewer free radicals. The atmospheric alterations produce secondary pollutants that also have hazardous properties or potential toxicity. The formation of the secondary pollutants will vary depending on atmospheric conditions. A comprehensive assessment of the health risks posed by DE would also consider the risks posed by the atmospheric reaction products, a task that is not addressed in this

assessment. Table 12-1 lists many of the particle-phase and gaseous-phase emissions in DE, as well as the atmospheric reaction products associated with each of these emissions.

12.2. HAZARD ASSESSMENT

Hazard assessment reviews what is known about the ability of DE to cause adverse effects (i.e., toxicity) in humans and laboratory animals and characterizes the likelihood that these effects are, in fact, human hazards. It also discusses the biological mechanisms that may be causing the toxicity and comments on the reliability and uncertainties of key studies.

12.2.1. Hazard Assessment for Health Effects Other Than Cancer

As reviewed in Chapter 5, exposure to diesel exhaust has been shown to induce a number of effects in humans and in experimental animals. As exposure progresses from episodic to more frequent, from shorter and longer duration, or from small to large concentrations, the evidence shows that symptoms progress from being annoying to being more temporarily disabling and become more severe, with increasing likelihood of permanent damage at high enough or long enough exposure. Although this section sets the stage for drawing conclusions about the potential for hazard, quantitative evaluations to estimate acceptable exposure levels are discussed in Chapter 6, the Inhalation Reference Concentration section.

12.2.1.1. *Effects From Acute Exposure*

The most readily identified acute noncancer health effect of DE on humans is its ability to elicit complaints of eye, throat, and bronchial irritation as well as physiological symptoms such as headache, lightheadedness, nausea, vomiting, and numbness and tingling of the extremities. Such symptoms have been reported by individuals exposed to DE on busy city streets or in bus stations. Recent human and animal studies also show that acute DE exposure episodes play a role in the development of allergic disease (immunological allergic reactions), resulting in prolonged hypersensitivity to DE and perhaps other ambient contaminants. We do not know what DE concentrations, per se, induce allergic responses, though particle concentrations of 10^6 per cm^3 induced symptoms of eye and nasal irritation and airway resistance in one study.

Acute animal studies have also shown that the gaseous components of DE elicit toxic responses. These include NO_2 (lung damage) and aliphatic aldehydes (irritation). Other animal evidence shows that acute exposure to high enough concentrations of whole DE does cause lung damage. In laboratory animals acutely exposed to high concentrations of whole DE, pulmonary edema (an excessive accumulation of fluid) often occurs during the first few days of exposure. After several days, aggregations of particle-laden macrophages have been observed in the

Table 12-1. Particle-phase and gaseous-phase emissions from diesel exhaust and their atmospheric reaction products

Emission component	Atmospheric reaction products
Particle-phase emissions	
Elemental carbon	—
Inorganic sulfate	—
Hydrocarbons (C_{14}-C_{35})	Little information; possibly aldehydes, ketones, and alkyl nitrates
PAHs (≥4 rings) (e.g., pyrene, benzo[*a*]pyrene)	Nitro-PAHs (≥4 rings); nitro-PAH lactones
Nitro-PAHs (≥3 rings) (e.g., nitropyrenes)	Hydroxylated nitro derivatives
Gaseous-phase emissions	
Carbon dioxide	—
Carbon monoxide	—
Oxides of nitrogen	Nitric acid, ozone
Sulfur dioxide	Sulfuric acid
Hydrocarbons	
Alkanes ($\leq C_{18}$)	Aldehydes, alkyl nitrates, ketones
Alkenes ($\leq C_4$) (e.g., 1,3-butadiene)	Aldehydes, ketones
Aldehydes	
Formaldehyde	Carbon monoxide, hydroperoxyl radicals
Higher aldehydes (e.g., acrolein)	Peroxyacyl nitrates
Monocyclic aromatic compounds (e.g., benzene, toluene)	Hydroxylated and hydroxylated-nitro derivatives
PAHs (≤4 rings) (e.g., phenanthrene, fluoranthene)	Nitro-PAHs (≤4 rings)
Nitro-PAHs (2 and 3 rings) (e.g., nitronaphthalenes)	Quinones and hydroxylated-nitro derivatives

Source: Health Effects Institute (1995).

alveolar regions of the lungs. At the same time, a proliferation of cuboidal type II alveolar cells is observed, replacing thinner type I cells and thickening the alveolar walls. Because of the key role alveoli play in the exchange of gases, these changes may inhibit the efficiency of pulmonary function. Human studies are inadequate to evaluate potential toxic effects resulting from acute exposures.

The observed effects from single high-dose or episodic exposures in test animals are consistent with effects seen with lower and more long-term exposures. This suggests that total accumulated dose may be one basis for characterizing DE hazards, but this could be too simplistic for all aspects of DE toxicity because dose-rate aspects cannot be ruled out.

12.2.1.2. *Effects From Short-Term and Chronic Exposure*

Based on suggestive evidence from human occupational studies, combined with multiple controlled laboratory animal studies in several species, a high level of confidence exists that chronic exposure to DE constitutes a noncancer respiratory hazard for humans. As DE exposure levels and duration increase, the onset of respiratory symptoms in humans is observable on a limited basis, whereas in animal studies the onset of symptoms is more clear and replicable. Current data do not support confident identification of health hazards other than for the respiratory system. Chapter 5 discusses this topic in more depth.

Several studies of workers occupationally exposed to DE on a short-term basis have monitored pulmonary function at the beginning and end of work shifts to see if this marker of respiratory distress has been impaired by exposures. Short-term symptoms are seen (e.g., bus garage workers experienced burning and watering of the eyes, coughing, labored breathing, chest tightness, and wheezing), but no reduction in pulmonary function. A study of stevedores showed an adverse effect on pulmonary function, but normal function returned a few days after DE exposure stopped. It was noted in one study that occupationally exposed smokers appeared to demonstrate larger work-shift respiratory function decrements and increased incidence of respiratory symptoms. Other occupational studies did not find statistically significant effects from short-term exposure, though these studies have limitations that reduce the ability to detect responses.

Noncancer effects of chronic DE exposure have also been evaluated in epidemiologic studies of occupationally exposed workers (metal and nonmetal miners, railroad yard workers, stevedores, and bus garage mechanics). Some of the data indicate an absence of increased respiratory disease associated with exposure. In a few studies, though, a higher prevalence of respiratory symptoms, primarily cough, phlegm, or chronic bronchitis, was observed among the exposed, but usually without significant changes in pulmonary function. However, two studies,

one of stevedores and one of coal miners, detected statistically significant decrements in baseline pulmonary function consistent with obstructive airway disease, thus providing some suggestion that impairment of pulmonary function among occupational populations may be occurring. Recent investigations have also indicated that human exposure to DE may result in the development of immunologic-driven allergic hypersensitivity; this would be a new health concern for DE exposure.

Overall, these results are suggestive of adverse chronic effects for humans, but database limitations preclude drawing more definitive conclusions. The epidemiologic investigations suffer from methodological limitations that confound the observations and limit the assignment of these observations to DE exposure. The limitations include (1) incomplete information on the extent of exposure to DE, necessitating in some studies estimations of exposures from job titles and resultant misclassification; (2) the presence of confounding variables such as smoking or occupational exposures to other toxic substances (e.g., mine dusts); (3) the short duration and low intensity of exposure; and (4) unlike in animal experiments, pathological evaluation of human lung tissue is seldom available for confirmatory analysis.

The suggestive human experience, however, is reinforced by a considerable body of animal evidence that clearly correlates DE exposure with pulmonary injury. The combined human and animal data are sufficient to infer that this hazard likely exists for humans. Short-term animal exposures to DE containing high concentrations of particulate matter (PM) resulted in histological and cytological changes in the lungs, but only minimal effects on pulmonary function. A number of long-term laboratory studies with rats, mice, Chinese hamsters, Syrian golden hamsters, cats, and Cynomolgus monkeys found varying degrees of adverse lung pathology. Exposures for several months or longer to levels markedly above environmental ambient concentrations resulted in accumulation of particles in the animal lungs and an impaired ability to clear particulate matter from the lungs. Histological studies also showed a variety of changes in respiratory tract tissue, including focal thickening of the alveolar walls, replacement of Type I alveolar cells by type II cells, and fibrosis. Because these effects were seen in a wide range of animal species, there is a compelling basis to believe that humans would also be at hazard under some condition of exposure.

Respirable particles in general have been implicated as etiologic factors in various types of chronic human lung diseases (U.S. EPA, 1996). Ambient particulate matter (PM) is associated with increased morbidity and mortality, aggravation of respiratory and cardiovascular disease, changes in lung function and increased respiratory symptoms, changes to lung tissues and structure, and altered respiratory defense mechanisms. The effects vary as one considers "fine" particles (e.g., $PM_{2.5}$) compared with "coarse" particles (e.g., PM_{10}). The vast majority of DE

particles are fine and ultrafine in size and thus contribute to ambient levels of $PM_{2.5}$. In addition, DE contributes significantly to total ambient PM. For instance, Yoshizumi (1989) indicated that for Tokyo, the yearly mean concentration of DE particles was about 40% of the total particle concentration.

Epidemiologic studies of the effects of DE on organ systems other than the pulmonary system are scant. Animal studies have suggested that liver and kidney changes may be occurring at high concentrations, along with some indication of neurotoxic effects. Impaired growth rates have also been observed in animals chronically exposed to DE. However, since these effects are only seen at relatively high exposure levels, this does not imply a hazard for humans at low ambient exposures.

12.2.2. Toxicity Mode of Action

An understanding of the mode of action(s) (MOA) for toxicity allows one to make informed choices about how to translate observed toxicity data into specific risk assessment guidance that protects human health. For example, MOA information may help answer several questions: (1) Are all humans equally susceptible or just some population subgroups? (2) Are animal responses predictive of potential human responses because the animal MOA is thought to operate in humans? (3) Are high-dose effects extrapolatable to low ambient levels of exposure, and what does the shape of the low dose-response curve look like? and (4) a number of other specific qualitative and quantitative matters. In the absence of convincing or reasonably clear MOA information, scientific inference or default assumptions based on science policy are used in order to facilitate risk assessment conclusions, if a hazard potential is suspected.

Chapters 7, 9, and 10 contain more in-depth review of the mode-of-action topic.

With DE being a mixture and having several distinct components, the topic of MOA(s) is complex. For the carbon core particle component of DE, the pathogenic sequence following the inhalation of the diesel particle begins when alveolar macrophages (AMs), "scavenger" cells that defend the lungs from invading foreign matter, ingest diesel particles. When AMs ingest particles in large numbers they are activated and release chemical signals that attract neutrophils (a component of white blood cells) and additional AMs. As the lung burden of diesel particulate matter increases, particle-laden AMs aggregate in alveoli adjacent to terminal bronchioles. The overloaded neutrophils and AMs produce and release compounds that mediate inflammation. The particle-laden macrophages also become less mobile, thus decreasing their ability to clear particles from the lung. The latter series of events may result in inflammation of the lung, with replacement of very thin type I alveolar cells with more cuboidal type II cells, slowing exchange of oxygen and carbon dioxide. Continued exposure may result in further consequences, such as lung fibrosis and/or emphysema. This mode of action has a dose threshold because at some point

the normal detoxification mechanisms become overloaded and consequential toxicity ensues. This MOA may also contribute to a carcinogenic response as discussed below. At least in the rat, the particle-driven toxicity and carcinogenicity can be viewed as being driven by the elemental carbon particle or a combination of the physical particle and the accompanying organics, depending on how several types of studies are interpreted. Intratracheal instillation of unaltered DE particles gave a stronger carcinogenic response than particles stripped of the organics, suggesting that particle-associated compounds play a role in tumor induction. Inhaled pure carbon particles (printex), however, were as effective in the induction of lung tumors as DE. Printex has a larger surface area per unit mass than diesel particulate matter. An initial notion that organics play an insignificant role is not necessarily indicated, however, because the very large surface area of the printex, a factor known to be related to cancer potency, may well make up for its lack of surface-adhering organics. In fact, a recent study has shown that stripping the organics from the DE particulates reduced their carcinogenic potency. Several animal studies also show that filtered DE (e.g., the gases without the coated particles) is ineffective in producing lung tumors in rats.

As for the chemical compounds that coat the particles, mutagenicity and genotoxicity assays reveal activity for the intact coated particles as well as organics extracted from the particles. Some of the gaseous fractions of the DE mixture are also mutagenic. The correlation of mutagencity-genotoxicity with carcinogencity isn't rigid, but the presence of this activity gives rise to support for a carcinogenic hazard, as well as suggesting some possible modes of action for cancer.

The operable mode of action for toxicity of the DE mixture taken as a whole is not definitively known. There are likely to be several modes operating, given the many DE constituents. A simplistic combination of modes to consider might have the following elements: (1) DE organics at low or high doses can initiate DNA damage which, if particles were not present, would have a probability to advance to cancer as a function of increasing total dose. This would also be considered a likely nonthreshold process because of the mutagenic properties of many of the organics on the particles and in the gases, and by EPA preference would be modeled for extrapolation purposes with a linear model; (2) with particles also present that overload normal lung detoxification at higher exposures, the particles continue to deliver organics to the deep lung, resulting in an increased residence time for the organics. The particles also add a second and seemingly dominant MOA (at least in the rat model) that induces a particle-specific proliferation of cells (because of the inflammatory consequences of the particles), thus accelerating growth of any DNA-damaged cells; (3) the presence of particles, even at nonoverload levels, may also influence carcinogenicity by contributing to additional DNA damage via the free

radicals present on the particle surface, which are potent oxidizing agents for bio-organic substrates.

The observation that some of the DE-induced rat tumors are different pathologically from what one would expect in humans and that some are similar may also support the thought that multiple MOAs may be present.

Given the information at hand, dual modes of action are more likely than a single mode. One would be driven by particles and one by the organic/inorganic components, with a shifting influence as the exposure/dose varies from high to low. The role of contributory toxicity from other constituents, such as NO_2 or oxygen free radicals, among others, is uncertain.

It would be ideal if the MOA were informative about whether all humans were equally susceptible or whether some population subgroups were more or less susceptible. The human evidence, i.e., occupational and population-derived studies, does not provide any particular insight about variations in susceptibility, nor do the animal studies. Neither female versus male nor adult versus children's susceptibility differences are specifically indicated: the former may have been discernable given the nature of the studies, but there is no study basis to address possible children's risk issues. Later, in this section, a qualitative discussion about susceptibility is included that acknowledges that toxicological wisdom suggests that background respiratory system conditions could make some in the population more susceptible to chronic effects of DE exposure. Similarly, infants and children could have greater susceptibility simply because their developing organs and defense systems may be less effective at dealing with insults from DE exposure. These suggestions are not unique to DE exposure, though.

12.2.3. Carcinogenic Hazard Assessment

For inhalation exposure, both human studies and animal bioassays are available to assess the chronic exposure carcinogenic potential for DE. In fact, both the human and the animal studies provide evidence that exposure to DE has potential to be carcinogenic to humans under some condition of exposure. Chapters 7 and 8 review these data in detail. A finding about the hazard potential does not specify the magnitude of the impact, information on which is discussed in Chapter 11, the carcinogenicity dose-response evaluation section.

12.2.3.1. *Human Evidence*

A total of 26 key epidemiologic studies have been evaluated to examine the association between exposure to DE and increased cancer response. The positive human evidence consists of observed increases in lung cancer mortality in a number of occupational exposure studies and some suggestion of other possible cancer sites. Cohort, case control, and population-based studies

are available. Exposure is most often defined indirectly by occupation or job title in the industry-related studies and is self-reported in population-based studies. The lack of direct exposure measurements, a condition common to retrospective epidemiology studies, is an overall limitation in the database. An excess risk (e.g. elevated standardized mortality ratios, relative risks, or odds ratios >1.0) for lung cancer was observed in 5 of 9 cohort studies and 8 of 10 case-control studies. Of these studies, three cohort and three case-control studies observed a dose-response relationship by using duration of employment as a surrogate for dose.

The most convincing evidence that exposure to DE can induce lung cancer in humans comes from case-control and cohort studies among U.S. railroad workers and truck drivers. The study of railroad workers, a well-conducted and well-analyzed study, is evaluated and published as both a cohort and case-control study with varied controlling for confounders. The case-control study is the best for control of confounders, especially the question of smoking and its possible role as a confounder for the reported lung cancer increase. Statistically significant higher risks of 41% to 43% for lung cancer were found in the case-control study for 20 or more years of exposure, and these risks were not confounded by smoking or asbestos exposure, adjustments for which were rigorously accounted for in the study methodology. In the retrospective cohort study of these same railroad workers, the risks varied from 20% to 72% higher than the general population, all statistically significant depending on the duration of exposure. Although adjustments for possible asbestos exposure were accounted for, there was no adjustment for the possible role of smoking. However, recognition was given to the rigorous smoking adjustments in the case-control study, which showed no effect on risks. Though the overall risks were increased in the railroad worker cohort study, the identification of a dose-response relationship is a subject of debate. A case-control study of truck drivers showed statistically significant increased risks of 80% to 240%, depending on data stratification and duration of exposure after adjustment for smoking.

There is a notable consistency in finding elevated, although not always statistically significant, increases in lung cancer among workers exposed to DE in several industries. There are industry-specific findings of elevated lung cancer risk from truck drivers, professional drivers, and railroad workers, with some of the studies having adjusted for smoking. When the possible role of smoking as a confounder was accounted for, the increased risks prevailed.

A very recent meta-analysis (Bhatia et al., 1998) shows the consistency of elevated risks in the epidemiology database and lends clear support to a causal association between increased risks for lung cancer and exposure to DE. Using 29 epidemiology studies, 23 of which met inclusion criteria, statistically significant relative risks (RR) for all studies were 1.33 (95% CI = 1.24-1.44). A subanalysis of case-control studies showed a RR of 1.33 (95% CI = 1.18-1.51); for

studies that specifically controlled for smoking, the results were nearly identical. These findings play an important role in analytically showing the trend of the evidence across much of the epidemiology database. The quantitative findings of aggregate relative risks may also be useful for dose-response analysis, if individual study RRs are not thought to be suitable for some reason.

The relative risks from all studies with elevated risks, statistically significant or not, are on the low side, generally < 2. Several of the best individual studies have RRs in the 1.4-1.7 range. Low relative risks are harder to interpret as being definitive because of the possibility that unresolvable uncertainties could be responsible for or could be influencing the elevated risks. When only one or two positive studies with low RRs exist, there is some uncertainty about whether the inference of an effect is the proper interpretation, compared to a case in which several studies have low RRs, where the uncertainty is less and the inference that a real effect is being seen is more confident. With several low-RR diesel studies available and the meta-analysis clearly demonstrating the increased RR pattern, the low-RR situation would not discount the inference about an effect from exposure.

In most risk assessment situations positive epidemiologic findings are weighed within a framework of "causality" criteria. The causality framework, with its related biological factors and physical factors, helps to rationalize the increased epidemiologic responses within a broader context of plausibility. When these criteria are applied to the positive DE studies, all of them apply well. Other assessors using similar criteria may come to a different conclusion because there is no rigid attainment measure within the causality criteria.

With all evidence and analysis taken into account, it is concluded that the human evidence is "highly suggestive" of an association between DE exposure and lung cancer in retrospective occupational settings. This is just short of a more definitive finding of a "known" human carcinogen, primarily because of deficiencies in exposure information.

Human study evidence for other forms of cancer is inconclusive.

12.2.3.2. *Animal Evidence*

Animal studies show that DE is carcinogenic in test animals, which in the simplest sense raises a concern that DE has a carcinogenic potential in humans. The direct inhalation exposure rat studies are best for direct observation of inhalation responses. Chronic-exposure animal studies conducted prior to the 1980s did not use inhalation as the route of exposure; instead, exposure was artificially produced by lung implantation, intratracheal installation, subcutaneous and intraperitoneal injection, or dermal application. Of note here is that organic extracts from the particles, as well as the whole particles (with the absorbed organics), are carcinogenic in many of the older artificial exposure route assays.

When focused on the newer inhalation studies and recognizing the reproducibility of results, one concludes that if exposures are adequate, inhalation of diesel exhaust will induce lung cancer in rats and, under some conditions, in mice, albeit at higher concentrations than in rats. Generally, rats showed significant increases in lung tumors beginning at exposures of 2,200 $\mu g/m^3$ or higher (the human equivalent concentration [HEC] for 2,200 $\mu g/m^3$ is about 700-900 $\mu g/m^3$). These exposures are higher than those thought to be present in the human occupational studies (i.e., 125 up to 500 $\mu g/m^3$), which in turn are higher than the ambient exposures of interest for humans (e.g., 0.6-3.6 $\mu g/m^3$). Along with the increased tumor incidence in the rat studies, there was evidence of particle-induced inflammation and particle clearance overload in the lung. Exposures to rats below 2,200 $\mu g/m^3$ did not elicit an observable lung cancer response, while the accompanying evidence of inflammation trailed off more gradually as dose was lowered below 2,200; some inflammation was still seen at 1,000 $\mu g/m^3$. Inhalation of diesel exhaust has also induced significant increases in lung tumors in a few, but not all, strains of female mice; exposure concentrations of 6,000 $\mu g/m^3$ or greater were needed to see a significant response. Although responses were not detected in rats or mice at lower exposure concentrations of 350-2,200 $\mu g/m^3$, these studies with nominally 50 animals probably lack the sensitivity to reveal a threshold or a response to a less potent mode-of-action-driven carcinogenic process. Attempts to produce positive responses by inhalation exposure in Syrian golden hamsters, cats, or monkeys were unsuccessful. The negative results in cats and monkeys may be explained by an inadequate exposure duration (only 2 years) in these longer-lived species, whereas hamsters are known to be less sensitive to lung tumor induction compared to rats and mice.

There is convincing evidence, based on numerous published studies, that DE constituents are also mutagenic or, in a broader sense, genotoxic and/or carcinogenic. This supports the concern for a carcinogenic hazard for humans as well as suggesting possible mode-of-action and related approaches for low-dose risk estimation. The whole particle, particle extracts, and gaseous portions of diesel exhaust have been shown to cause changes in genetic material, which is not surprising because each component contains one or more mutagenic constituents. Human studies show increased formation of DNA adducts with DE exposure, and cultured human cells show an increased occurrence of sister chromatid exchange. Extensive studies with *Salmonella* have unequivocally demonstrated mutagenic activity in both particulate and gaseous fractions of DE. The induction of gene mutations has been reported in several in vitro mammalian cell lines after exposure to extracts of diesel particles. Particles have also induced structural chromosome aberrations and sister chromatid exchanges in mammalian cells. The question of germ-cell interaction, however, and the potential for human germ-cell mutagenic risk from exposure to diesel emissions remain unanswered.

12.2.4. Weight-of-Evidence Summary

A conclusion about the likelihood of a human carcinogenic hazard involves a weighing of the evidence from the human and animal studies, mode-of-action information, and evidence from ancillary studies. Scientific uncertainty or debates about some aspects of the evidence as well as different guidance about how to weigh evidence can easily lead to modestly different conclusions. EPA uses Carcinogen Risk Assessment Guidelines to frame its approach to weighing evidence and judging the likelihood of hazard. Overall, EPA considers that the human evidence for DE gives a clear signal about the likely presence of a human hazard. Subtle deficiencies in the human data influenced EPA in holding back a definitive call for a "known" human carcinogen based on the human data alone. Other conclusions that give a high likelihood to DE being a human carcinogen are also defensible. Animal evidence clearly shows that at high experimental exposures, DE produces lung cancer. Numerous DE particle extracts have been shown to pose carcinogenic hazards to animals and thus probably humans, even though some artificial exposure routes are used and carcinogenic responses are not necessarily in the lung. When mode-of-action information is considered, valid questions are raised about whether the lifetime rat bioassay, per se, is a good model for defining the hazard for humans at low exposures. Still, many of the individual components of the DE mixture are known or thought by the health science community to potentially pose a carcinogenic hazard. Supporting evidence for mutagenicity-genotoxicity in human cell cultures and in various other in vitro test systems for the whole DE mixture, the particle coatings, and the DE gases also support a likely mammalian-based carcinogenic hazard potential. For low levels of exposure, the hazard is hypothesized to be related to the organics present, while at high doses the presence of particles seems to exert a definite influence. Everything considered, EPA believes that DE is "very or highly likely" to be carcinogenic in humans by the inhalation route. This discussion and finding is consistent with the provisions of EPA's Proposed Cancer Risk Assessment Guidelines (U.S. EPA, 1996).

DE is considered to be a "probable" human carcinogen by inhalation exposure and to best fit into cancer weight-of-evidence category B1 according to EPA's 1986 Cancer Risk Assessment Guidelines (U.S. EPA, 1986). This conclusion evolves from positive yet "limited" evidence in the human studies, a "sufficient" level of evidence in bioassays, and consideration of the supporting information from mutagenicity and genotoxicity data.

In comparison with other agents classified into this category, the confidence in the DE weight-of-evidence call, on a relative scale of high, medium, or low, is on the high side. The conclusion that DE is a "probable" or a "very likely" rather than a "known" human carcinogen is due to several subtle limitations of the human studies, including the difficulty of reconstructing reliable estimates of exposures many years in the past, the lack of systematic quantitative records

of ambient air quality, and the inability to completely eliminate confounding factors such as exposure to other pollutants, especially tobacco smoke, and the small increases in relative risk that make possible confounding more critical. The weight of the evidence for a human health hazard is nevertheless only just shy of being sufficient to view DE as a "known" human hazard, and for this reason "very likely" may be a better descriptor than "probable."

Once the hazard potential for humans is identified, information about the possible impact of the hazard (i.e., measures of risk and risk estimation, etc.) is treated as a related but separate dose-response issue. A complete conclusion about carcinogenicity thus has two components.

12.3. DOSE-RESPONSE ASSESSMENT

Dose-response assessment focuses on the relationship of exposure/dose to a biological response and how the response might change with dose; it also investigates the translation of this relationship to human low-exposure circumstances. The response(s) are the ones previously identified as important in the hazard assessment, and the low-dose aspects are approached by extrapolation, if appropriate, from an observable response range to lower exposure/dose levels, such as ambient levels of interest. Key dose-response assessment choices are influenced by definitive knowledge and informed reasoning about the mode of action. In the absence of such information, assumptions (i.e., defaults) are used, some of which may be conservative. Chapter 11 contains a more detailed review of dose-response issues.

12.3.1. Considerations in Modeling the Dose-Response Relationship

In order to know the extent to which a substance poses a hazard to human health, it is necessary to estimate the magnitude or incidence of adverse effects induced in humans by a particular concentration or dose of a substance. Since intentional experimentation with suspected toxicants on humans is not acceptable, animal studies are usually a major source of information about possible human hazards, yet the animals may have a variety of differences from humans that could affect the translation and extrapolation of the response. Animal studies usually involve exposure to high concentrations of toxicants, so that dose-response for low exposures must be inferred via informed assumptions and extrapolation. Even studies of humans are often at higher exposures than most individuals would experience at ambient conditions, so that conclusions about the effects of lower levels of exposure once again require assumptions and extrapolation.

Dose-response analysis of the positive human data may be approached in any of several ways, depending on what the data allow. One almost always has to struggle with exposure uncertainties when using retrospective human studies, as well as with questions of confounding exposure from other carcinogens, such as from smoking, when lung cancer is involved.

Statistically significant mortality increases for lung cancer, in this case in cohort or case-control studies, may be useful analytical starting points. Adjustments in exposure dosimetry for humans wouldn't be needed, as is the case with animal studies.

In the DE dose-response analysis based on animal studies, several issues about exposure-dose to the lung can be reasonably accounted for because of available dosimetry research and modeling. The issues include species differences in particle deposition efficiency, particle clearance rates, lung surface area, respiratory rates, and transport of particles to lung-associated lymph nodes. Another set of issues involves the suitability of the rat model for assessing human risk from the standpoint of biological sensitivity as well as exposure-related MOA even after dose adjustment to equivalent concentrations. A final series of issues involves the choice of dose-response extrapolation model to be used (i.e., threshold or nonthreshold, linear or nonlinear, and biologically based or curve-fitting models).

While whole DE has multiple components, the various gaseous/semivolatile components of DE are in rough proportion to the particle concentration. Particle concentrations have been adopted by researchers to document whole DE mixture exposure and hence are used in this assessment as a dosimeter in evaluating carcinogenic and other adverse effects. For DE, the critical site of action is in the lower respiratory tract, particularly the alveolar region of the lung. The exposure route of concern for humans is inhalation, which is also the method of exposure used in some of the available experimental data, though important information from animal or other in vitro assays is gleaned from studies where the exposure is not related to inhalation per se but to artificial experimental exposures.

12.3.2. Dose-Response Assessment for Health Effects Other Than Cancer

A considerable body of evidence clearly shows a major noncancerous health hazard may be presented to the respiratory system following inhalation of DE. Based on pulmonary function and histopathological and histochemical effects, a rough estimate can be made concerning what chronic dose/exposure rates of DE (measured in terms of the concentration of diesel particulate matter) can be observed to cause an adverse effect and which exposures do not; this then is a starting point for establishing adequate margins of safety. A reliable experimental database and established EPA dose-response evaluation methods have been used to derive an inhalation reference concentration (RfC) for chronic exposure to DE as a guide in determining a level of long-term exposure that is thought to have an acceptable margin of safety from hazard for chronic exposure adverse effects other than cancer.

12.3.2.1. ***Derivation of an Inhalation Reference Concentration***

The derivation of an inhalation reference concentration (RfC) for DE is a dose-response approach used by EPA for noncarcinogenic, threshold chronic effects. An RfC is defined as an estimate of a continuous inhalation exposure to the human population, including sensitive subgroups, with uncertainty spanning perhaps an order of magnitude, that is likely to be without appreciable risks of deleterious noncancer effects during a lifetime. The RfC approach is based on the assumption that a threshold exists for the human population below which no effect will occur.

As an alternative to using a no-observed-adverse-effect-level (NOAEL) in the RfC methodology as a dose-response marker, this assessment examined a benchmark dose/concentration (BMC) analysis approach, a newer approach that EPA has used to derive the RfCs for carbon disulfide, chlorodifluoromethane, and several other chemicals (U.S. EPA, 1995). The BMC refines the ascertainment of the NOAEL.

The database EPA chose to work from for RfC derivation consisted of 10 long-term (greater than 1 year) studies of inhalation of diesel engine emissions in laboratory rats. The available human studies, as discussed earlier, were qualitatively suggestive of adverse effects but were inadequate for RfC consideration. The selected rat studies were conducted by the Inhalation Toxicology Research Institute (ITRI) and the Japanese Health Effects Research Program (HERP). These studies were selected because each identified respiratory effects after chronic exposure and provided good information about pulmonary histopathology. Further, the selected studies spanned a wide range of exposures, from 350 to 7,000 $\mu g/m^3$ with three exposures in the 350-960 $\mu g/m^3$ range. Human equivalent concentrations were calculated using a dosimetry model developed by Yu et al. (1991) that accounted for species differences in respiratory exchange rates, particle deposition efficiency, differences in particle clearance rates at high and low doses, and transport of particles to lymph nodes.

The adopted RfC comes from the HERP study, which showed a NOAEL of 460 $\mu g/m^3$ (human equivalent concentration, HEC, = 155 $\mu g/m^3$). While particle overload is thought to be still present at 1,000 $\mu g/m^3$ to some degree, at 460 $\mu g/m^3$ the overload is thought to be much less, if not minimal. Consistent with standard RfC practice for a good chronic animal study, two types of uncertainty factors were used to further lower the NOAEL-HEC to a value having a sufficient margin of safety for humans. An uncertainty factor of 3 out of 10 was used to account for interspecies sensitivity; that is, humans could be somewhat more sensitive than rats. Out of a possible factor of 10, credit is given to the dosimetry adjustment procedures used. We would also note that some researchers believe rats could be more biologically sensitive than humans to DE particles, but we do not know whether rats are more or less sensitive to the organics. A second

uncertainty factor of 10 is used to account for sensitive members within the human population, this being standard practice unless mechanistic or other data suggest otherwise. The resulting total uncertainty factor is 30. With 155 $\mu g/m^3$ divided by 30, the resulting RfC is:

RfC = 5 $\mu g/m^3$ of diesel particulate matter (DPM).

A BMC analysis also supports the use of an RfC of 5 micrograms per cubic meter of air. The derived RfC is considered reliable because of the high quality of the animal studies. Additional support for this RfC level is provided by a retrospective analysis of an earlier monkey study in which monkeys were exposed to DE at a concentration of 2,000 $\mu g/m^3$ for 2 years. Two years would be considered subchronic in this longer lived species. On the basis of minimal effects on the lungs of the monkeys, it could be argued that the exposure level is either a NOAEL or a marginal LOAEL (lowest observed adverse effect level). After adjustment to the equivalent human concentration and using appropriate uncertainty factors, an RfC value similar to that derived from the rat can be shown.

It is interesting to note that EPA's recently adopted particulate matter ($PM_{2.5}$) standard, with an adequate margin of safety, is 15 $\mu g/m^3$, as a 3-year average, based on human studies. The noncancer effects from DE are qualitatively similar to some of those for $PM_{2.5}$, though particle differences exist, as do the presence of absorbed organics and gases. A comparative discussion of the $PM_{2.5}$ standard and the DE RfC is not currently pursued in this assessment.

12.3.3. Dose-Response Assessment for Carcinogenic Effects

Evidence shows that DE is likely to induce lung cancer by the inhalation route of exposure, though the mode of action is imperfectly understood. Using a range of studies, some assumptions, extrapolation models, and animal-human dosimetry factors appropriate for DE, it is conceptually possible to estimate human cancer potency as a function of lifetime exposure assuming that DE is a human carcinogen.

12.3.3.1. *High- to Low-Dose Extrapolation and Mode-of-Action Considerations*

Because the biological mechanisms that result in cancer may be different for DE at low and high doses, a good understanding of the MOA would be needed in order to generate DE-specific, biologically based probability models for extrapolating from high to low doses. Currently, however, there are significant gaps in understanding of the mode of action(s) by which DE produces cancer, and thus the development of a rigorous DE-specific model is not pursuable. Given this imperfect situation, the risk assessor falls back on making choices about modeling,

some of which can be informed choices while others are more public-health-driven policy choices.

To recount the mode-of-action discussion earlier, at low to high doses the DE cancer hazard is believed to have at least two modes. One involves mutagenic/genotoxic mechanisms associated with the organic substances adsorbed to DE particles as well as those in the gases. The organics include substances that are known to be mutagenic and/or carcinogenic in animal bioassays. Superoxide or hydroxal free radicals on the surface of fresh DE particles may also contribute to this mode of action. The mutagenic organics are present in approximate proportion to the particulate concentration, which likely varies somewhat by diesel engine circumstances. These substances could initiate cells or be complete carcinogens. A second mode involves nongenotoxic particle-specific mechanisms associated with lung particle overload, which is likely to dominate at high doses, according to rat studies, by exacerbating the promotion of initiated cells. The influence of the particles at low, nonoverload exposures may be limited to delivering organics, but this is an unknown.

If we favor the organics (gases or absorbed organics on the particles) as a likely etiologic agent for a suspected low-exposure carcinogenic hazard, the issue of bioavailability of the organics is an important consideration. There would be little doubt about the bioavailability of the gaseous-phase compounds in the DE mixture; whether they present a large enough dose to make a carcinogenic response observable is a different question. The bioavailability of the organics coating the particles is a more complex topic. Rat studies using radiolabeled BaP and nitropyrene-coated carbon particles have shown that lung retention time of the organic is significantly increased compared to a nonparticle instillation and that the gradual elution of BaP, for example, was faster than the lung clearance of the carrier particle itself. An understanding of the role extracellular fluids play in extracting the organics from the particles is unclear. Extraction of the organics by alveolar and other cell types is theorized but not well understood. At least in the rat and mouse models, a carcinogenic response was not seen below the whole-DE exposure levels that were also associated with particle overload, implying that a dose adequate to cause tumors was not present. On the other hand, one recent bioassay (Dasenbrock, 1996) has shown by intratracheal instillation that rat tumor yield is decreased (from 17% to 4%) when diesel particulates are stripped of their organics. Overall, limited data make it plausible to assume that there is a gradual release of organics from the particles, with resulting exposure to the respiratory epithelium.

Ideally, the dose-response curve that would be used to extrapolate from high to low exposures would reflect changes in the MOAs, a result of the possible transition from primarily nongenotoxic modes of action at high exposures to primarily genotoxic ones at lower exposures.

However, data needed to construct this unifying dose-response curve are lacking. While the exposure levels at which primarily nongenotoxic MOAs may predominate can be inferred from lung pathology in rats (e.g., Chen and Oberdoster [1996] say the transition range probably lies in the >100 to 1,000 $\mu g/m^3$ exposure range for rats), the change in slope of the dose-response relationship can only be crudely approximated by the use of a biologically based model, some of whose parameters are estimated, or by estimating low dose risk from nonsignificant responses. In general, experimental studies inherently lack sensitivity for estimating dose-response in the low-dose range, except for extremely potent carcinogens, and DE seems not to be that potent. Use of human data to estimate low dose risks is also pursued, but this is not as robust as one would like because of the underlying exposure uncertainties and low relative risks limit, which for some assessors limits the confidence in the human-study-based risk estimates.

High- to low-dose extrapolations from various types of animal studies have been pursued using a variety of modeling concepts, all of which have low-dose linearity because of the inference of mutagenicity/genotoxicity. A variety of approaches is useful when information is inadequate to make a clear or reasonable call about the mode of action or when definitive insight into dose-response aspects is lacking. The margin of exposure (MOE) is also investigated for additional insight about the magnitude of extrapolation.

12.3.3.2. *Estimating the Cancer Risk of Diesel Exhaust*

In attempting to determine the cancer risk (i.e., potency) of DE, several kinds of studies and dose-response approaches were considered, including relative risks from epidemiologic studies, animal bioassay responses, a comparative potency approach, and the use of a biomarker, B[a]P, as a dosimeter.

12.3.3.2.1. ***Assessing risk using epidemiologic studies.*** As an ideal, epidemiology data are preferred for risk estimation if the available data are rigorous enough. One quantitative approach is to identify and use the dose-response relationship from an epidemiology study. This has been a debatable issue with the best of the epidemiologic studies, the cohort study of railroad workers by Garshick et al. (1988). This was a well-designed study with one of the largest cohorts. Because of the ongoing debate regarding the dose-response, EPA sees no benefit to generating more analysis of the issue until the cohort study can be updated or consensus can be reached about how to best use the current railroad worker cohort data. Garshick et al. (1987) also published a nested case-control study of the railroad workers, which showed increased relative risks of lung cancer from DE exposure. These can be used to back into a risk derivation by using a proportional population risk approach and overlaying this with separate assumptions about average exposure.

For convenience EPA has started with a published proportional population risk analysis (McCellan, 1989) based on the Garshick case-control study relative risks. Additionally, EPA selected a reasonable exposure estimate of 125 $\mu g/m^3$ (and also included 500 $\mu g/m^3$ for comparison), corrected the numerical risk estimates for occupational versus ambient exposure, and thus back-calculated equivalent estimates of unit cancer risk. The resulting risk estimates have 95% upper and lower bounds as well as MLE values.

The adoption of a particular exposure value for risk derivation from the railroad worker study is a critical choice since risk magnitude is directly proportional to the exposure. The true exposure of the railroad workers beginning in the late 1940s–1980s period is an unknown, though Woskie (1988a, b) in conjunction with the railroad worker epidemiology study, evaluated current levels of exposure for the railroad worker job categories. Woskie sought to evaluate the current exposures and comment on historical exposures for railroad workers by job category and did so by collecting limited personal monitoring data for the job categories as well as employing modifying factors to account for a number of influencing factors. The job category exposure estimates for the late 1980s showed that geometric mean exposures might range across all job categories from about 17 μ/m^3 to 134 $\mu g/m^3$ at a 95% confidence level, these included an adjustment for cigarette smoke. It was also suggested that exposures were approximately constant over the 1950s to 1980s given the circumstances of railroads that were sampled in the study. National projections were slightly higher at 31–35 $\mu g/m^3$ up to 125–157 $\mu g/m^3$. Woskie mentions that anecdotal reports suggest that exposures were likely higher in the early period versus the later years due to, for example, minimal ventilation, but the magnitude of difference could be ascertained.

From these crude approximations, one has to make a choice as to how appropriate overall estimates of 125 or 500 $\mu g/m^3$ are. Woskie's work would suggest that 125 is a reasonable choice and that 500 seems too high. Woskie's estimates were for worker shifts, while EPA's interest would be for a 24-hour exposure which could be a lower value. The diesel locomotive engines went through two generations, the first generation lasting through the 1950s, with a second generation starting to appear in the 1960s. Woskie's data was mostly derived from first generation engines that were still in use in the 1980s and which probably were higher emitters of DPM. The selection of 500 $\mu g/m^3$ has no particular support, though it can't be ruled out. It does show the sensitivity of the risk calculations to a fourfold higher (125 vs 500) exposure estimate.

An exposure estimate of 125 $\mu g/m^3$ is in general agreement with measurements made during the period of the underlying railroad worker study. As there is indirect evidence, but few actual measurements, indicating that historical occupational exposures were higher, the use of 125 $\mu g/m^3$ is a reasonable conservative choice, though not without uncertainty. The selection of a best

exposure value is an unresolvable question, though reason would suggest that the true exposures are in this range.

For each exposure, three estimates of risk can be provided: an upper end, an MLE (maximum likelihood estimate), and a lower end. The case- control, proportional risk study-based estimates define a range as follows:

Exposure = 125 $\mu g/m^3$:	Upper end risk	200×10^{-5} per $\mu g/m^3$
	MLE is	100×10^{-5}
	Lower end risk	10×10^{-5}
Exposure = 500 $\mu g/m^3$:	Upper end risk	50×10^{-5}
	MLE is	30×10^{-5}
	Lower end risk	3×10^{-5}

Upper bound from biomarker (BaP)	~ 1×10^{-5} per $\mu g/m^3$ (as discussed in next section, MLE = 0.26×10^{-5})

EPA is aware that the authors of the proportional risk analysis may have some reservations about their conclusions using the case-control study, given the dose-response debate about the larger cohort study, but EPA risk assessors believe the merit of using the case study relative risks is not diminished by the cohort study debate.

A few comments about the human databased estimates are important to the question: How good are they? The average occupational exposure of 125 $\mu g/m^3$ is unlikely to be large enough to induce lung particle overload in humans (a human level of 500 $\mu g/m^3$ is likely to be in the overload range, extrapolating from rat studies). When using an average estimate for exposure, we must remember that about half the time the exposures are higher, and while noting that occupational exposure occurs about 40 h per week, the long-term occupational exposure is less than lifetime, and thus mean particle concentrations adjusted to continuous exposure are unlikely to exceed 100 $\mu g/m^3$ and may be notably lower. Because cancer induction at nonparticle-overload conditions is reasoned to be influenced by the mutagenically active organic fraction and perhaps by the oxygen radicals present in fresh exhaust, the use of linearized low-dose extrapolation is a supportable modeling approach. Individual smoking data were obtained in the Garshick et al. (1987) case-control study, as was information about possible asbestos exposure, thus eliminating a major potential source of bias. Also, relative risk ratios from other diesel epidemiologic studies did not differ greatly from the 1.41 (CI = 1.06, 1.88) reported in the Garshick et al. (1987) study, increasing confidence in the Garshick-based relative risk findings.

For example, in a recent meta-analysis of 34 studies by the State of California (Cal-EPA, 1997a) relative risk ratios ranging from 1.12 to 1.43 were derived, depending on the model used and whether or not they were corrected for smoking.

Exposure estimation is a notable uncertainty in the risk estimates derived from the railroad worker case control study. However, according to Woskie et al. (1988), the 125 $\mu g/m^3$ estimate was probably reasonable as an average exposure near the end of the study period. The EPA risk estimate of 200×10^{-5} per $\mu g/m^3$ defines the high end of a range of plausible upper-bound estimates for DE cancer risk, while 3×10^{-5} $\mu g/m^3$ defines the lowest end of the human data range. The MLEs of the two exposure scenarios define a tighter range of $30\text{-}100 \times 10^{-5}$ per $\mu g/m^3$.

12.3.3.2.2. ***Assessing risk using a biomarker.*** A second approach using human data is the use of a biomarker as a dosimeter. Pike and Henderson (1981) related the concentration of benzo[a]pyrene (B[a]P) to smokers, British gas workers, U.S. coke oven workers, U.S. hot pitch workers, and residents of rural and urban locations and found good agreement in predicting lung cancer risk. They concluded that while B[a]P is not the only carcinogen present in DE, and perhaps not even the most important, it is a reasonably accurate dosimeter for assessing risk from combustion or pyrolysis of petroleum products or tobacco and could therefore be appropriately used for DE risk assessment. Based on Pike and Henderson's estimated lung cancer risk of 1/1,500 per ng/m^3 B[a]P and a reported B[a]P concentration of 3.9 ng per μg of diesel particulate matter in exhaust from a Volkswagen engine (Heinrich et al., 1995), a maximum likelihood estimate of lung cancer risk of 2.6×10^{-6} per $\mu g/m^3$ of diesel particulate matter can be derived. The 95% upper bound of this value, while not calculated by the authors, is near 1×10^{-5} per $\mu g/m^3$.

A strength of the biomarker approach is its moderately good accuracy in estimating cancer risk from exposure to a number of combustion/pyrolysis pollutants using B[a]P as a dosimeter. However, while most of the combustion products assessed in the study contain organics similar to DE, unlike DE they have little or no insoluble particulate matter. Although this approach, like the comparative potency method, fails to account for the carcinogenic effect of the particle itself or for modifications in potency because of the association of the organic component with particles, the human exposures of interest presumably don't have particle-driven effects either. Of course, the B[a]P concentration might also vary in diesel samples because of different types and sizes of engines being run under varying conditions and using different fuels.

12.3.3.2.3. ***Assessing risk using animal studies.*** Previous EPA attempts to quantitatively estimate cancer risk based upon chronic rat bioassays used some form of a linearized model to

extrapolate risk to low doses. This approach was based upon the assumption that cancer response is a direct function of exposure concentration at all exposure levels. As discussed previously, this assumption is no longer supported by the available data because of the apparent difference in mode of action at high versus low exposures. At high concentrations, rat lung cancer is believed to be induced by an interaction of particle overload with associated pathology, carcinogenic organics, and possibly oxygen free radicals. At lower doses, only the latter two are likely to have a major impact. This is likely to result in a dose-response curve that is nonlinear at least in the high-dose region. Unfortunately, the animal bioassays lack sensitivity, in particular, the number of animals per group are too small to directly measure low-dose responses or show clearly where the mode of action transition occurs on the dose-response curve. Clearly, at exposures above 2,200 $\mu g/m^3$ the dose response would be nonlinear. At some exposure level below 2,200, the particle driven mode of action is diminished. For environmental levels of exposure, the risk estimation objective would be to partition the dose-response curve and develop risk estimates from the low-exposure portion.

Several quantitative modeling approaches were used with animal data to gain some insight about low exposure only risks—none were completely satisfactory. The simplest approach is to derive a 95% upper-bound estimate of risk using the highest concentrations of DE not inducing pathologic responses and lung cancer. This conceptually places an upper bound on risk using elevated but not statistically significant responses. The low-dose groups from the Mauderly and Ishinishi studies (i.e., concentrations < 500 $\mu g/m^3$) are suitable for such an analysis. Combining these studies increases the statistical power somewhat, though the variability statistics of the minimal response would tend to increase the risk estimate. The resulting risk estimate using a linear multistage extrapolation model (LMS) is 19×10^{-5} per $\mu g/m^3$. This is several fold higher than LMS estimates using all (including the high-dose responses) exposure groups (about 3.4×10^{-5}) but approaches the higher risks suggested by the human data.

An alternative approach (mentioned in EPA's *Proposed Guidelines for Carcinogen Risk Assesment*) involves identifying a point of departure on the dose-response curve, perhaps ED_{10} or ED_{01}, the concentrations inducing carcinogenic responses in 10% or 1% of the animals, and then extrapolating risk from that point as long as extrapolation was justifiable. This was done for the rat data, both ED_{10} and ED_{01} risks were estimated using an LMS model. The answer was nearly identical to the 3.4×10^{-5} obtained from using the entire dose-response data. The linear model seems insensitive for this particular data set.

A third approach published by Chen and Oberdörster (1996) involved development of a biologically based dose-response (BBDR) extrapolation model. They applied it to the rat dose-response data to consider the risk sensitivity to a threshold-particle driven MOA compared with a

nonthreshold-mutagen driven MOA. If particle effects are assumed at all concentrations, i.e., no threshold for particle effects, then the model predicts risk virtually identical to those predicted by the linearized multistage model. If a threshold for particle-induced cancer effects is assumed at 1,000 $\mu g/m^3$, the BBDR risk estimate is about fivefold lower at a concentration of 1 $\mu g/m^3$. This difference is consistent with the hypothesis that organics and oxygen radicals play a more important role as concentration becomes lower. The results, however, only suggest the influence of mutagenic-nonthreshold versus mutagenic-threshold modes of action, because data are insufficient to validate the model.

There is also some debate, and hence uncertainty, regarding the biological adequacy of the rat as a model for evaluating any human risk. This begs the question whether rats may be uniquely sensitive to particle-induced cancer, perhaps because of their different respiratory tract anatomy. Only two other rodent species have been adequately tested, hamsters and mice. The response in mice was equivocal, and negative in hamsters. Hamsters, which do not respond to DE exposure, are resistant to induction of lung cancer from DE. Since there is evidence for human carcinogenicity of DE in epidemiologic studies, one could argue that the rat may be more qualitatively similar to humans in response to this agent than are the other laboratory species. Furthermore, rats have been shown to respond similarly to humans when exposed to cigarette smoke (Finch et al., 1995). Although the rat could be more sensitive from a particle standpoint, we wouldn't necessarily say the same in regard to the organic components, which are playing a role as well. Thus, while the use of rat data does involve uncertainty, it is not justifiable to fully discount the rat as a plausible model for establishing a range of possible human risk from exposure to DE.

A strength of rat-based risk estimates is that the studies are of good quality and provide tumor responses that are in essential agreement with one another. Another strength is the ability to use a sophisticated dosimetry model to derive equivalent target tissue concentrations between rats and humans. However, with all statistically significant rat responses occurring at exposures where particle overload is also occurring, the use of rat data to define possible risk in any manner has distinct shortcomings and resulting uncertainties. For reference purposes, an LMS-derived averaged risk estimate of 3.4×10^{-5} per $\mu g/m^3$ was calculated from the high-dose rat responses of Mauderly, Ishinishi, and Brightwell. Use of measured rather than modeled lung particle burdens from the Mauderly et al. (1987) study did not significantly affect results. As a matter of curiosity, if dose equivalence is based on lung burden per unit body weight$^{3/4}$, a more traditional means of species extrapolation for organics, LMS risk estimates would increase about threefold.

As it turns out, all of the rat-based risk estimates are near the low end of estimated risks.

12.3.3.2.4. ***Assessing risk using a comparative potency method.*** A third approach, developed before either animal bioassay or human epidemiologic data became available, is the comparative potency method of Albert et al. (1983). In this method, exhaust particle extracts obtained from three light-duty engines, manufactured by Nissan, General Motors, and Volkswagen, and a Caterpillar heavy-duty engine were evaluated for potency in a variety of short-term tests. The ratios of potencies of these extracts were then multiplied by the unit risk estimates of related combustion or pyrolysis products, such as roofing tar, cigarette smoke condensate, and coke oven emissions, for which unit risk estimates based on human data had already been derived. The potency ratio of DE to each of these was multiplied by their unit risk estimate. Using this method Albert et al. (1983) derived a unit risk estimate for DE averaging about 3×10^{-5} per $\mu g/m^3$.

The comparative potency method was developed more than 15 years ago because bioassay data were lacking at the time and because it was believed that cancer induction was a function of the organic components. Conceptually, this approach can still be of use in rationalizing lower limits on risk. However, one must accept the assumption that relative potency in short-term tests will be similar to relative potency for lung cancer induction. Any reluctance may be balanced by considerable reliance on dermal exposure in some of the short-term studies. Since both skin and lungs are epithelial tissues, confidence that relative potencies are similar increases. Another possible weakness in the comparative approach is a failure to account for potential differences in the relative bioavailability of the organic fraction during exposure to whole exhaust versus exposure to particle extracts, or the possibility that association of organics with particles may alter their effectiveness. It is not known whether these issues raise significant concerns.

Tabular summary of animal-based risk estimates

19×10^{-5} per $\mu g/m^3$	Upper limit from low-dose rat studies
3.4×10^{-5} per $\mu g/m^3$	Upper bound from high-dose rat responses
3×10^{-5} per $\mu g/m^3$	Upper bound from BaP comparative potency analysis
1×10^{-5} per $\mu g/m^3$	Linear extrapolation using BBDR model

12.3.3.3. ***Bounds for Cancer Risk and Margin of Exposure***

Each of the dose-response and risk derivation approaches, using human or animal data, has known uncertainties that seemingly preclude selecting one as the "most scientifically valid" or best estimate. Taken collectively, the approaches provide a numerical basis for defining a range of plausible upper-bound risks, upper-bound meaning that conservative assumptions (e.g., linear low-dose extrapolation) have been used and this is not likely to result in an underestimate of risk.

For the reasons cited earlier, the risk estimate of 2×10^{-3} per $\mu g/m^3$ from human studies is the highest estimate and thus defines the upper-end value for a range of estimates. Three different animal-based approaches that assumed that lung cancer at low doses is a function of the organic components (the comparative potency method, the biomarker method, and low-dose estimation of risk from nonsignificant rat response data) resulted in upper-bound risk estimates from near 1×10^{-5} up to 19×10^{-5} per $\mu g/m^3$. A BaP human biomarker-based estimate of 0.26×10^{-5} as an MLE also exists, with an upper bound of the MLE being about 1×10^{-5}. Since no threshold is assumed for the organic components of DE, if particles do contribute to risk at low exposures, the upper bound may not be as conservative as would normally be the case, i.e., normally we would say the true risk, which is not definable, could be between the upper bound and be as low as zero. The 1×10^{-5} per $\mu g/m^3$ risk estimate from various animal-based approaches seems to be a floor of all estimates and therefore, establishes the low end for a range of risks.

The upper-end risk estimate of 2×10^{-3} per $\mu g/m^3$ from the epidemiologic study is based on an assumed long-term average exposure of 125 $\mu g/m^3$. If a higher exposure were chosen the risk estimate would decrease by a proportional amount. Risk estimates based upon animal bioassays, comparative potency, and the biomarker approach are as much as 200 times lower. The range of possible human risks encompasses mixed MOA assumptions and does not fully discount any experimental animal model system. Given the presence of known uncertainties with the adequacy of the various animal test systems as a predictor of human hazard, and with the uncertainties for the railroad worker exposures, neither the human nor animal test system provides a scientifically compelling basis for selection of a single best risk estimate. Therefore, a range of plausible risk estimates is recommended to characterize the possible public health impact of exposure. Public health policy preferences might be appropriate, such as recognizing that the human data-based estimates at least avoid uncertainties of species extrapolation and biological relevance associated with the animal estimates, and thus a preference for the range of human data-based risk estimates would be reasonable. An argument also could be made that the human estimates derived from 125 $\mu g/m^3$ exposure have more support than 500 $\mu g/m^3$ because Woskie's assessment was more consistent with a level of 125. Some may also favor the MLE risk estimates from exposure assumptions of 125 and 500 as a preferable selection of risks.

Upper bound risks ranging from 1×10^{-5} to 200×10^{-5} (i.e., 2×10^{-3}) per $\mu g/m^3$ are recommended for bounding the risk of human lung cancer induced by exposure to DE. The risk estimates evolving directly from human data run from 3 to 200×10^{-5} per $\mu g/m^3$, with a subrange of maximum likelihood estimates spanning 30-100 $\times 10^{-5}$ per $\mu g/m^3$. Animal-based estimates range from 1 to 19×10^{-5} per $\mu g/m^3$.

It may be insightful to consider the margin of exposure (MOE) for these risk estimates. An MOE compares ambient exposure of interest to LOAELs (adjusted to human equivalent LOAELs) from the human and from the animal studies. The resulting margin shows the extrapolation range from, e.g., an observable cancerous effect level to an ambient human exposure of interest. The margin value illustratates the range of extrapolation that has occurred when risk models have been employed to predict dose-response below the observable data. The greater the magnitude of extrapolation, i.e., the margin, the more general uncertainty there is. If 2 $\mu g/m^3$ is an ambient human DE exposure of interest, if an estimate of 125 $\mu g/m^3$ is selected from many possible choices for the human study of railroad workers, and 3.5 mg/m^3 is a LOAEL rat exposure from the Mauderly rat study (human equivalent concentration: 0.36 mg/m^3 = 360 $\mu g/m^3$ after adjusting to continuous exposure conditions and across species), all of the human equivalent exposure information needed for an MOE comparison is available. What is seen is that the range of extrapolation from the human studies is about 1/3 of that for the animal studies, thus suggesting a reduced margin for uncertainty compared to animal estimates. The ambient levels of interest are nevertheless 10-60 times lower than the lower of the exposure scenarios associated with the human studies.

The MOE comparison is displayed graphically in Figure 12-1. This figure may provide a clearer picture of the relationship between exposure and various risk estimation recommendations coming from this assessment.

12.3.4. Susceptible Subgroups

The hazards previously identified, i.e., acute symptoms including exacerbation of asthma, and chronic effects such as reduced pulmonary functions and other respiratory weaknesses, are assumed to be possible consequences in individuals of average health and in their adult years. Individuals with preexisting lung burdens of particulates may have may have less of a margin of safety from DE hazard consequences, though this cannot be quantified. In reality, DE exposure is probably additive to many other minute or larger exposures to mutagenic organics and particulate matter, but the magnitude of this additivity has not been estimated in this assessment. For example, adults who predispose their lungs to increased particle retention (e.g., smoking or high particulate burdens from nondiesel sources), have existing respiratory or lung inflammation or repeated respiratory infections, or have chronic bronchitis, asthma, or fibrosis (e.g., silica exposure) would have a much lower margin of safety and thus would be at greater hazard from DE exposure. It hasn't been shown per se in DE studies, but infants and children may have a greater susceptibility to the acute/chronic toxicity of DE for the conventional public health reason

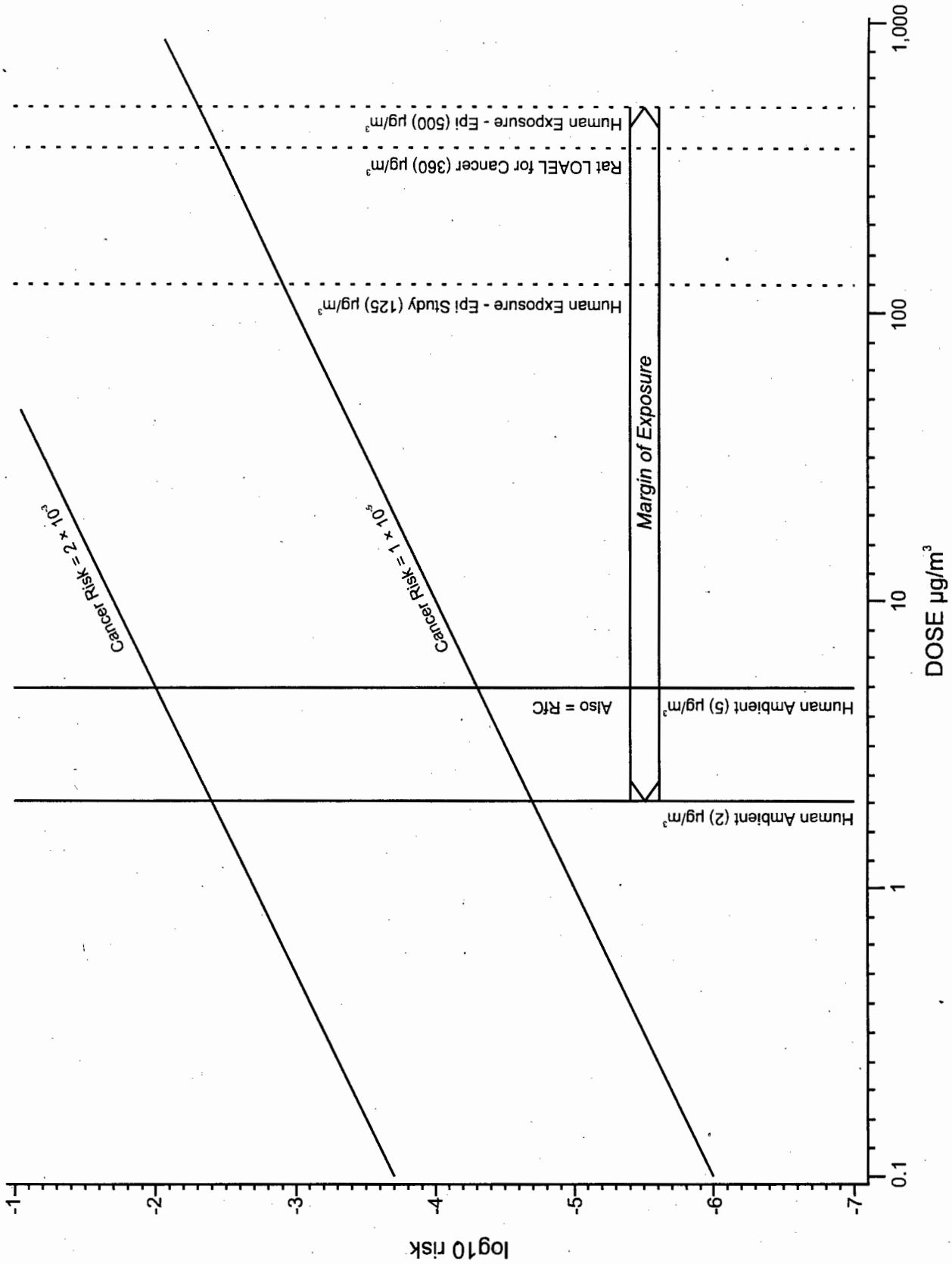

Figure 12-1. Comparison of cancer risks, RfC, and MOE.

that their developing pulmonary and immunologic systems may be more susceptible than an average adult's.

12.3.5. Other Comprehensive Diesel Exhaust Health Assessments in the United States

In 1997 Cal-EPA (California Environmental Protection Agency) released a draft hazard and risk assessment for DE emissions. Cal-EPA concluded that a reasonable, very likely explanation for the increased risks of lung cancer seen in experimental animal and epidemiologic studies is a bona fide causal association between DE exposure and lung cancer. Because of evidence for both carcinogenic and noncarcinogenic toxic effects, they also proposed to list DE as a toxic air contaminant in California. Their conclusion for the carcinogenic hazard is similar but not identical to EPA's, (i.e., DE is highly likely to be carcinogenic in humans). Cal-EPA also provided a range for cancer risk per unit of lifetime exposure that is virtually identical to EPA's risk range. The Cal-EPA risk range included a subrange of estimates from the animal studies and a subrange of estimates from the human studies, as has EPA in this assessment. EPA indicates that DE is "highly likely" to be a human carcinogen, whereas Cal-EPA is slightly more certain about the likelihood. Given the health data uncertainties, data gaps, and the discretionary choices that are made in risk assessment, the differences in the EPA and Cal-EPA assessment findings are not significant. Cal-EPA's recommendations for noncancer respiratory hazards are identical to EPA's because they adopted the EPA RfC of 5 $\mu g/m^3$. As of February 1998, the Cal-EPA assessment was still in draft.

12.4. EXPOSURE PERSPECTIVE

Diesel emissions are complex mixtures containing thousands of organic and inorganic constituents in both gas and particulate phases with differing chemical reactivities. After entering the atmosphere, they are transported and transformed according to their distinctive characteristics, undergoing physical and chemical changes that may form secondary pollutants more harmful than their predecessors. Thus, a knowledge of diesel emissions at or near their sources is not sufficient to fully assess their impact on human health and welfare. However, data on how DE contributes to exposure levels for these secondary pollutants are currently lacking.

Determining the amount of DE present in the ambient air is also complicated by the difficulty of distinguishing organic compounds and particles that originate in diesel engines from those that originate in gasoline engines or come from other sources. This source speciation is not well sorted out in the ambient characterization of DE.

Nonoccupational exposure to DE is worldwide in urban areas, with lesser exposure in rural areas. Certain working populations are also exposed to higher levels of DE than the rest of the

population. The level of exposure will differ within geographic areas based on the number and types of diesel engines in the area, as well as atmospheric patterns of dispersal and the location of the individual relative to the emission sources.

While a detailed exposure assessment for DE has not been conducted as part of this study, the following exposure data are provided to give some context for the hazard assessment and dose-response analysis.

Estimates of annual average concentrations of particulate matter in the ambient air, published in Chapter 9 of EPA's Motor Vehicle-Related Air Toxics Study (U.S. EPA, 1993), may be used to generate a crude estimate of the concentration of particulates from diesel exhaust in the ambient air. The total concentration of particulates from DE in ambient air in urban areas for 1995 was estimated as 2 $\mu g/m^3$, the concentration in rural areas as 0.6 $\mu g/m^3$, and the nationwide average concentration as 1.1 $\mu g/m^3$.

In an alternative estimation, using ambient monitoring data, total suspended particulate matter (TSP) for 1990 was determined to equal 48 $\mu g/m^3$. With approximately 5% of total particulate matter associated with DE, multiplication of the total by the fraction contributed by diesel exhaust and adjusting for time spent indoors results in an integrated estimate of 1.5 $\mu g/m^3$, according to EPA's hazardous air pollution exposure model (HAP-EM, 1988).

Exposure estimates for more highly polluted locations are somewhat greater. Estimated mean concentrations of DPM for Los Angeles were reported to be 2.7 $\mu g/m^3$ (Sienicki and Mago, 1992). McClellan (1986) estimated concentrations on urban freeways and street canyons to be as great as 15 $\mu g/m^3$.

Recent Cal-EPA (1996) studies show winter period estimates in three California locations for diesel PM_{10} of 4 to 22 $\mu g/m^3$. A broader Cal-EPA analysis shows average ambient outdoor diesel PM_{10} to range from 0.2 to 3.6 $\mu g/m^3$ across 14 California air basins with a population-weighted average of 3.2 $\mu g/m^3$. Concentrations in occupational settings may be higher.

Recent studies, including a study of the Baltimore Harbor Tunnel (conducted by the Desert Research Laboratory for the American Petroleum Institute) and an ORD measurement study of tailpipe emissions from a moving heavy duty diesel truck, have confirmed that dioxins are formed and emitted from heavy-duty diesel trucks. ORD's draft dioxin source emission inventory, being developed as part of the ongoing Dioxin Reassessment effort and scheduled to undergo peer review in April 1998 estimates that 60 g TEQ are emitted from U.S. trucks. When this estimate is compared with total estimated U.S. emissions of 3,000 g TEQ, it appears that diesel trucks are not a major dioxin source. However, it is unknown whether such emissions could have significant local impacts, since current information does not permit us to rule out the possibility of exposures of interest. The types of exposures that have been of particular concern

from stationary dioxin sources, e.g., incinerators, have been noninhalation exposures associated with ingestion of certain foods, e.g., beef and vegetables, contaminated by the deposition of stack-emitted dioxin. There is a potential for deposition of stack emissions from trucks to soil and water adjacent to some highways resulting in similar impacts. We recommend that appropriate data be collected.

The changing composition of DE (i.e., older engines vs. newer technology ones, heavy-duty vs. light-duty, and engines run under varied operating conditions) gives rise to questions about how the health data and the risk assessment findings in this report, which are based on pre-1998 engines, can be applied to present-day engine exhaust emissions and resulting ambient exposures. This is a complex question that is not rigorously addressed in this assessment. It is clear that newer technology engines will have somewhat different emission composition (i.e., perhaps reduced NO_x with increased fine particles), not to mention emission controls, which would reduce certain exhaust components, presumably larger particles. Since particle mass is the surrogate dosimeter used to correlate toxicity with exposure and public health impact, the implication is that we have a basis for scaling to account for exhaust changes. This relationship may be too simplistic, and thus further investigations of current-day emissions, and how they average out across the fleet or stationary engine use, may be warranted.

12.5. DIESEL EXHAUST HEALTH RISKS—A PLAIN-LANGUAGE OVERVIEW OF KEY INFORMATION

This section reviews key information about diesel emission hazards and risks by posing and answering simple questions. It is mostly duplicative of information found earlier in this chapter, though some added explanation has been added for background purposes.

What is diesel exhaust (DE)? What happens when it enters the environment?

Diesel engines are very durable and have performance characteristics that make them a desirable alternative in certain uses. The diesel engine, whether it be in an automobile, truck, off-road equipment, locomotive, or ship, produces exhaust from the combustion of diesel fuel. The exhaust is a mixture of organics and inorganic constituents (i.e., products of incomplete combustion). The exhaust can be invisible or be seen as a gray or black smoke. When visible, what is seen is the particle fraction of the exhaust mixture, usually from an engine that is not required to control its emission or one that is not well maintained. In the simplest terms, DE is a mixture of carbon core particles that have a coating of various inorganic/organic compounds, as well as gases and semivolatiles. The identifiable organic and inorganic compounds number in the hundreds. The particles have a spongelike structure and a very large surface area per gram, which

make them an excellent carrier for adsorbed inorganic/organic compounds. The amount of specific chemical compounds present and the size of the particles depends on the engine design, load, operating speed, fuel consumption, and whether or not the engine is in a well-maintained state. The diameter of diesel particles is very small, typically 0.1 to 0.25 μm, with more than 75% of the particles smaller than 1 μm. Light-duty diesel engines emit 50-80 times and heavy-duty engines 100-200 times more particulate matter than catalytically equipped gasoline engines. The heavy-duty and off-road diesel engines, as a group, account for most of the DE particulate emissions discharged into ambient air.

When the exhaust first escapes to the ambient air it is called "fresh" exhaust, which also connotes some increased chemical and biological reactivity properties, compared to "aged" exhaust, which after a day or so has diminished reactivity. Once in the ambient air, some of the organic, inorganic compounds and oxygen free radicals associated with the fresh exhaust begin to transform into other chemical compounds because of their exposure to sunlight or other atmospheric elements. The exhaust mixture also becomes dispersed and thus diluted in the ambient air. The topography and/or climatic conditions in a particular area may promote dispersal, or in some cases hinder dispersal to the point of causing a slow accumulation of DE components, e.g., because of ground-level air stagnation. The particle fraction of DE contributes to the background ambient particulate matter (PM) in the air, while the various gaseous inorganic/organic components add to other background loadings in the ambient air.

The measurement of whole DE is complex because it is a mixture of particles and gases. The approach adopted by researchers has been to use particle mass as a surrogate for the whole mixture. The mass measurement is in units of weight (μg, microgram) per volume of air (m^3, cubic meter). This emphasizes the particle fraction of the whole DE mixture together with its adsorbed organic/inorganic components, rather than the gaseous organic/inorganic constituents. The latter are, however, in relative proportion to the particle mass present.

How are individuals exposed to DE? How does it enter and leave the human body? Is there a test to determine whether exposure has occurred?

Individuals may be exposed to DE when they are in an area where diesel engines are in use and the exhaust mixture is breathable. Diesel engines are nearly everywhere, so it becomes a matter of relative frequency of contact (i.e., exposure), duration of exposure, and concentration of the exhaust mixture. Some occupational settings may be prone to more frequent and higher exposures, such as in engine maintenance shops, heavy equipment operations, mining, or bus terminal operations, to suggest a few. A nonoccupational setting that may have a higher than average ambient exposure could be, for example, among those who spend a notable part of their

day in the vicinity of diesel roadway traffic, such as in or around highways or urban street canyons. For some DE emissions, one can see smoke or soot, indicating that some relatively large particles are present. Emissions with the more typical small-diameter particle are virtually invisible. The odor threshold, at least according to one study, is about 200 $\mu g/m^3$ of particulate, or greater.

This assessment does not determine exposure levels across the population, but some exposure estimates from various sources are noted to provide a frame of reference. For example, several evaluations show average rural and urban ambient levels of DE to be in the range of 0.6-3.2 $\mu g/m^3$. Recent California-EPA studies show winter-period estimates in three California locations for diesel PM_{10} (particulate matter < 10 um) of 4 to 22 $\mu g/m^3$. A broader Cal-EPA analysis shows average ambient outdoor diesel PM_{10} to range from 0.2 to 3.6 $\mu g/m^3$ across 14 California air basins with a population-weighted average of 3.2 $\mu g/m^3$. Concentrations in occupational settings may be higher.

DE exhaust most easily enters the body by breathing, though in some occupations portions of the exhaust may cling to skin or hair and thereafter possibly be ingested as a consequence of hand-to-mouth activity. By far, the major exposure pathway is from breathing.

Once inhaled into the nasal passage, some DE mixture components could be deposited or absorbed along the upper nasal and respiratory tract, but most of the mixture travels into the lungs where the particles and gases are deposited on lung tissue. The inhale-exhale pattern of breathing results in some exhalation of the particles and gases; the remaining particles stay in the lung until the body's natural defense mechanisms mobilize to clear them out. The remaining gases and organics/inorganics coated on the particles are eventually absorbed into the lung tissue, then into the bloodstream, and thereafter begin a process of normal detoxification by various body organs followed by removal from the body via urine and feces.

There is no single medical test to determine if a DE exposure has occurred. Many symptoms of episodic DE exposure are similar to symptoms caused by other agents or, in some cases, the onset of a common cold. Invasive sampling of particle deposits in the upper respiratory tract or lung could be done, yet such particles may not be readily distinguishable from particulate matter from other sources.

How does exposure affect human health and how certain are we about these effects?

One way to consider the possible harmful effects of DE is to consider acute exposure (i.e., episodic/infrequent contact) versus chronic exposure (i.e., fairly continuous over long periods of time, such as years). As the exposure frequency and/or duration of the contact increase, acute exposure and its effects give way to chronic exposure and its consequences. Most health studies

are designed to evaluate either acute or chronic effects. Another aspect of the exposure event is to realize that in a general sense *total cumulative exposure and the rate at which the exposure is received in some manner influences the nature and/or the extent of a harmful effect, this being a traditional toxicological concept not unique to DE.* This relationship is not always definable in a rigorous way, but is useful as a concept in relating exposure features to toxic consequences. Many DE components have a potential of being harmful because they are toxic at some exposure in their own right. A DE component may start out being toxic, may start out being nontoxic but be changed into a toxic substance by the body's defense mechanisms, or have no toxicity at all in any phase. The question is, do the concentrations in the DE mixture, when taken as a total, cause harmful effects?

The pure carbon core DE particle, the organics coating the particle, and the gas/vapor phase components of the mixture all have health study evidence that shows toxicity, and thus potential to be hazardous under some regime of exposure. Taken individually, both the particles and some of the chemical compounds can be irritants and cause inflammation in the respiratory system, or in larger amounts cause more permanent harmful effects. For example, among the many hydrocarbons found in DE, 19 of them are believed or known to pose a human carcinogenicity hazard, with the magnitude of the risk thought to be proportional to total exposure over a lifetime. It is not clear precisely what components of the DE mixture are key to causing the acute effects or the more permanent chronic effects and, in fact, most of the components may play a role, which may change as the human exposure changes from low to higher levels.

Effects of acute exposure on humans have not been systematically and comprehensively studied, but there are some symptoms seen, depending on the person and the concentration of DE. The symptoms are a biological response to irritation of human tissues. The symptoms range from no effect, to annoying and quickly passing effects, to effects that cause temporary impairment, up to and including symptoms that may indicate permanent impairment. e.g., from a very high one-time exposure. Examples at the lower levels include: headache, runny eyes and nose, or nausea, up to and including restricted breathing due to respiratory resistance (asthmalike response). Immunologic allergic reactions resulting in long-term hypersensitivity to DE and perhaps other ambient agents may also be possible, according to some very recent human and animal research. Animal studies have confirmed the irritational aspects of contact with DE and further suggest that high acute exposures may cause lung damage. The supposition is that different people have different levels of tolerance or susceptibility and that the higher the exposure, the more likely people are, in general, to experience an unpleasant symptom or perhaps have a more permanent adverse effect such as hypersensitivity or lung damage.

As the exposure instances increase, changing from episodic to more continuous and increasing from weeks to months to years, it is clear that too much exposure increases the likelihood of noncancer respiratory system damage or the risk of lung cancer, and thus we say DE at some level of chronic exposure poses a respiratory hazard for humans. Both human and animal studies provide evidence of this. But the human evidence specifically for diesel exhaust is not as clear as that from the well-controlled studies in test animals. As the total exposure over a lifetime increases, basic respiratory functions can be impaired, and there is a probability (i.e., risk) that lung tumors may appear later in life. Part of the permanent harm may be caused by the particle portion of the exhaust, part may be caused by the other inorganic/organic constituents on the particle or in the DE gases, or all of these may be interacting to influence the adverse outcome. With animal studies being conducted at high test exposures, and with the occupational human studies being somewhat lower in exposure but greater than ambient levels of DE, it is necessary to rationalize whether DE also poses a low-exposure hazard, whereas it is clear that at high exposure/doses it does. Plausible explanations supported by observations about adverse effects at higher doses are not matched with equal information about how effects may develop at low doses, and thus there is uncertainty about how to best estimate the low-dose hazards or risk. These questions are a current pursuit of health researchers. EPA also takes the position that chronic DE exposure, at high or low concentrations, is very likely to increase the hazard and risk of an adverse consequence.

Specific individuals inherently have varied susceptibility to these adverse outcomes, depending on whether they already have a weakened or compromised respiratory system, perhaps by smoking or having allergic or asthmatic symptoms. Although it is not demonstrated, one could hypothesize that episodic or frequent exposure of young children to DE could disproportionally increase their lifetime respiratory hazard or lifetime cancer risk, because damage early in life could increase their susceptibility, in addition to their having a longer period to accumulate exposure. Current data do not permit any more definitive explanations, nor is there a confident identification of additional human health hazards beyond those to the respiratory system.

What recommendations exist to protect human health?

This health assessment identifies the likely human health hazards and uses additional risk assessment tools to assist decision-makers in understanding what is important for protection of public health. These tools are in the form of concentration-based exposure-response relationships based on the identified hazard likelihood (i.e., respiratory damage or lung cancer). These relationships facilitate the rough estimation of acceptable/unacceptable exposures or health

impacts. Use of these measures requires caution and recognition of the biological and risk assessment uncertainties that are present.

For the acute effects of DE exposure, there is no specific recommendation from this assessment for a concentration not to be exceeded in order to avoid acute effects, because of an absence of sufficient data. Clearly, if acute symptoms are noted one would want to remove oneself from the locale as soon as practicable, if for no other reason than personal comfort. As the level of exposure increases, the acute symptoms can become more annoying and be indicative of temporary impairment. With the inherent variability of susceptibility and sensitivity in the human population, it is not possible to judge the outcome of a specific exposure incident. The same statements could generally be made about exposure to gasoline exhaust and many other agents as well.

For the chronic effect hazards, EPA believes that for many people, keeping long-term exposures at or below 5 $\mu g/m^3$ of diesel particulate matter provides an adequate margin of safety for noncancer respiratory hazards. This level also includes a 10-fold margin to account for variability in the human population. This is not an absolute demarcation of acceptable versus unacceptable exposure, since an order-of-magnitude range of uncertainty is thought appropriate for this recommendation. For practical purposes, the belief is that as the long-term average exposure concentration exceeds this value, the likelihood of respiratory distress increases. The 5 $\mu g/m^3$ value comes from test animals who experienced respiratory distress at higher experimental exposures to which a margin of safety (e.g., uncertainty factor) and animal-to-human equivalence factors have been used to derive the 5 $\mu g/m^3$ level for humans. It was necessary to depend on animal studies because the human database was not robust enough. While children should not, a priori, be assumed to be protected by adult recommendations, at this time there is no separate recommendation for children, except for the general wisdom to minimize exposure as much as possible.

For carcinogenic hazard and risk of cancer over a lifetime, EPA is recommending that exposure be viewed as likely to pose a risk at low levels, as well as high levels, and is offering a crude range of cancer risks per unit of lifetime exposure in order to gauge the public health acceptability of exposures. The risk values provide an upper bound to the possible human risk, rather than a true estimate; the true estimate is undefinable and could be much lower. A range of risk estimators was provided because the available cancer data had too many uncertainties to justify the selection of one scientifically best estimate. The risk range is thought to bracket the upper limits of possible risk, and these values would not likely underestimate the true risk.

Assuming that DE is a cancer hazard for humans, EPA believes that the cancer risks for DE are not likely higher than 1×10^{-5} to 200×10^{-5} per $\mu g/m^3$ of diesel particulate. These values

evolve from consideration of human occupational exposure-responses, several types of high-dose animal studies, and extrapolation to provide an appropriate estimate for low exposures. Extrapolation below the range of observation has uncertainty but is necessary from a public health perspective, because low-exposure cancer effects are often not detectable, yet low exposure hazards are expected for DE given our knowledge about the DE mixture components. Numerically, the risk estimates are the same as saying the probability of cancer could be, and is not likely higher than, 1/100,000 to 200/100,000 per microgram of diesel particulate matter per cubic meter of air ($\mu g/m^3$, an average particle concentration over a lifetime).

With exposure information, a risk assessor can make crude estimates of the highest possible impacts on a population, if such analysis is desirable. Under this type of evaluation, for an individual the development of cancer is a matter of chance. A person either gets cancer or doesn't by the end of their lifetime, but in the interim they have a probability (i.e., a risk) of getting cancer. Nationally, the lifetime risk of being diagnosed with any type of cancer is about 1 in 4 (1/4), and as a cause of death is about 1 in 5 (1/5). Cancer of the lung runs about 1 in 12 for males and 1 in 19 for females.

As an example of how the crude risk estimations can be informative, at a hypothetical human average lifetime DE exposure of 2 $\mu g/m^3$, there would be little likelihood of noncancer respiratory hazard because this is less than 5 $\mu g/m^3$, whereas the cancer risks are not likely higher than 2 /100,000 (1 in 50,000) up to about 400/100,000 (1 in 250). At a concentration of 5 $\mu g/m^3$, which is still presumed protective for respiratory effects, there remains an upper-limit cancer risk of 5/100,000 (1 in 20,000) up to 1,000/100,000 (1/100). It should be noted that as these all of these risks are upper bound, the true risk is unlikely to be greater and may well be less.

Are there other important considerations of DE exposure?

Particulates (i.e, particulate matter, PM) are a prominent constituent in DE. Breathing nonspecific PM is a public health concern in its own right, as evidenced by EPA's 1997 Ambient Air Quality standards for PM. When present, DE plays a role in contributing to ambient PM, especially $PM_{2.5}$ (PM less than 2.5 um in diameter). Diesel particulates are small; more than 75% of them can be less than 1 μm, which means that EPA's new $PM_{2.5}$ standard provides another health-based reference point. DE particulates are potentially a more toxic fraction in a $PM_{2.5}$ mixture because the smaller DE particles (<1 μm) can be deposited deeper in the lung. Because of their small size they also have a large surface area per unit mass and carry a coating of organic compounds with them. Though diesel particulates are associated with a carcinogenic hazard, this is not indicated, per se, for ambient PM exposure.

Older diesel engines emit higher levels of nitrogen oxides (NO_x) than do gasoline engines, and NO_x is also an ambient urban contaminant that EPA seeks to reduce because of its influence on ozone formation, formation of nitrate PM, acid rain, and the eutrophication of coastal waters. Some new engine design is focused on reducing the NOx.

Those individuals who already carry a significant burden of particles in their lungs or have weakened respiratory systems (e.g., from allergies, asthma, or other respiratory system inflammation) could be at higher hazard/risk. These special population subgroups are difficult to enumerate, but they do exist. These same individuals might also be more sensitive to a number of insults, such as general PM or gasoline engine exhaust or smog.

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Appendix A

Experimental Protocol and Composition of Exposure Atmospheres

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	U.S. Environmental Protection Agency				
Reference	Bhatnager et al., 1980; Campbell et al., 1980, 1981; Hyde et al., 1985; Moorman et al., 1985; Pepelko et al., 1980b, 1981; Pepelko, 1982b; Pepelko and Peirano, 1983; Plopper et al., 1983			Laurie et al., 1980; Laurie and Boyes, 1980, 1981	
Engine type	Nissan CN 6-33, 3.24 L, 6-cylinder			3.24 L, 6 cylinder	
Operating mode	Federal short cycle			Federal short cycle	
Fuel type	No. 2 diesel			No. 2 diesel	
Fuel sulfur	0.15%			0.15%	
Exposure regime	8 h/d, 7 d/week, 124 weeks			8 h/d, 7 d/week, 16 weeks	
Exposure conditions	Control	Exhaust - weeks 1-61	Exhaust - weeks 62-124	Control	Exhaust
Particle conc. (mg/m^3)	0.00	6.34 ± 0.81	11.70 ± 0.99	0.01	5.97 ± 0.17[b]
Particle size		90% < 1 μm by mass; 50% ≤ 0.3 μm by mass			
CO_2 (%)	0.04 ± 0.002	0.30 ± 0.04	0.52 ± 0.04	0.05 ± 0.00[b]	0.28 ± 0.01[b]
CO (ppm)	2.20 ± 0.50	20.17 ± 3.01	33.30 ± 2.94	1.86 ± 0.06[b]	19.20 ± 0.35[b]
NO_2 (ppm)	0.03 ± 0.03	2.68 ± 0.80	4.37 ± 1.19	0.03 ± 0.00[b]	2.51 ± 0.10[b]
NO (ppm)	0.05 ± 0.04	11.64 ± 2.34	19.39 ± 3.80	0.08 ± 0.01[b]	11.14 ± 0.43[b]
SO_2 (ppm)	0.03 ± 0.02	2.12 ± 0.58	5.03 ± 1.03	0.46 ± 0.02[b]	1.82 ± 0.07[b]
SO_4^{-2} ($\mu g/m^3$)	-	-	-		
Ozone (ppm)					
Aliphatic aldehydes (ppm)	0.00	0.177 ± 0.043	0.338 ± 0.057		
Formaldehyde (ppm)	0.00	0.106 ± 0.029	0.251 ± 0.059		
Acrolein (ppm)	0.00	0.025 ± 0.003	0.034 ± 0.009		
NH_4^+	-	-	-		
THC (ppm)	2.82 ± 0.50	7.93 ± 1.42	11.02 ± 1.04	3.22 ± 0.08[b]	7.29 ± 0.11[b]
PAHs					
Benzo(a)pyrene		15.9 μg/g extract			
Benzo(e)pyrene		28.6 μg/g extract			
Benzo(a)anthracene		53.8 μg/g extract			
Benzo(k)fluoranthene		77.8 μg/g extract (k+b)			
Fluoranthene		155.8 μg/g extract			
Pyrene		198 μg/g extract			
Phenanthrene		145.2 μg/g extract			
Chrysene		71.6 μg/g extract			
Perylene		3.5 μg/g extract			
Indeno(1,2,3-Cd) fluoranthene		10.9 μg/g extract			
Indeno(1,2,3-Cd) pyrene		14.8 μg/g extract			
Benzo(ghi)perylene		21.1 μg/g extract			

[a] All ± are S.D., unless specified otherwise.
[b] Standard error of mean values.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]				
Facility/Sponsor	U.S. Environmental Protection Agency			
Reference	Wiester et al., 1980			Pepelko et al., 1980a
Engine type	Nissan CN6-33, 3.24 L, 6 cylinder			3.24 L, 6 cylinder
Operating mode	California cycle, modified			California cycle, modified
Fuel type	No. 2 diesel			No. 2 diesel
Fuel sulfur	0.15%			
Exposure regime	20 h/d, 7 d/week, 4 weeks			20 h/d, 7 d/week, 4 weeks
Exposure conditions	Control	Exhaust	Exhaust - irradiated	Exhaust
Particle conc. (mg/m^3)	0.00	6.32 ± 1.31	6.83 ± 1.44	6.40 ± 0.36[b]
Particle size		0.1-1.0 μm		
CO_2 (%)	0.04	0.261 ± 0.01	0.25 ± 0.03	0.26 ± 0.008[b]
CO (ppm)	2.0	17.4 ± 2.5	16.7 ± 4.0	14.61 ± 0.90[b]
NO_2 (ppm)	0.07	2.3 ± 0.4	2.9 ± 0.7	2.13 ± 0.09[b]
NO (ppm)	0.11	5.9 ± 0.6	5.0 ± 1.2	6.13 ± 0.18[b]
SO_2 (ppm)	0.0	2.1 ± 0.8	1.9 ± 0.8	2.10 ± 0.21[b]
SO_4^{-2} ($\mu g/m^3$)	0.00	0.57 ± 0.12	0.57 ± 0.13	0.577 ± 0.019[b]
Ozone (ppm)	0.0	0.0	<0.01	
Aliphatic aldehydes (ppm)				
Formaldehyde (ppm)				
Acrolein (ppm)				
NH_4^+				
THC (ppm)	0.00	31.6 ± 2.3	26.1 ± 1.6	31.56 ± 1.25[b]
PAHs Benzo(a)pyrene				
Nitropyrene				

[a] All ± are S.D., unless specified otherwise.
[b] Standard error of mean values.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	U.S. Environmental Protection Agency					
Reference	Pepelko, 1982a			Lee et al., 1978, 1980		
Engine type	Nissan, 6 cylinder, 3.24 L			3.24 L, 6 cylinder		
Operating mode	California cycle, modified			California cycle, modified		
Fuel type	No. 2 diesel			No. 2 diesel		
Fuel sulfur						
Exposure regime	20 h/d, 7 d/week, 4 weeks			20 h/d, 9 weeks		
Exposure conditions	Control	Exhaust	Exhaust - irradiated	Control	Exhaust	Exhaust - irradiated
Particle conc. (mg/m^3)		6.40 ± 0.36	6.75 ± 0.39		6.32	6.83
Particle size (μm) MMD[b] (GSD)[c]						
CO_2 (%)		0.247 ± 0.003	0.244 ± 0.007	0.040	0.252	0.255
CO (ppm)		16.9 ± 1.1	16.1 ± 1.3	2.0	15.7	15.4
NO_2 (ppm)		2.49 ± 0.18	2.76 ± 0.15	0.07	2.19	2.73
NO (ppm)		5.71 ± 0.21	4.53 ± 0.15	0.11	5.85	4.94
NO_x (ppm)						
SO_2 (ppm)		2.10 ± 0.21	1.86 ± 0.21		2.13	1.91
SO_4^{-2} ($\mu g/m^3$)		577 ± 19	569 ± 19	0.0	0.57	0.57
O_2 (%)						
Ozone (ppm)						<0.01
Aliphatic aldehydes						
Formaldehyde (ppm)						
Acrolein (ppm)						
NH_4^+						
Hydrocarbons (ppm)		31.6 ± 3.8	26.1 ± 3.4	2.0	15.6	15.0
PAHs Benzo(a)pyrene						
Nitropyrene						

[a] All ± are standard errors of weekly means.
[b] Mass median diameter.
[c] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]				
Facility/Sponsor	National Institute for Occupational Safety and Health			
Reference	Castranova et al., 1985; Fedan et al., 1985; Hahon et al., 1985; Lewis et al., 1986, 1989; Mentnech et al., 1984; Vallyathan et al., 1986			
Engine type	Caterpillar 3304, 7 L, 4-cycle			
Operating mode	8-mode mining cycle, 60% idling			
Fuel type	No. 2 diesel			
Fuel sulfur	0.34%			
Exposure regime	7 h/d, 5 d/week, 104 weeks			
Exposure conditions	Control	Exhaust	Coal dust	Exhaust + coal dust
Particle conc. (mg/m^3)			4.98 ± 0.82	3.23 ± 0.60
Respirable particles[b] (mg/m^3)		1.95 ± 0.25	2.00 ± 0.41	2.02 ± 0.30
Particle size (μm) MMD[c] (GSD)[d]		0.23 (± 2.5)[e] 0.36 (± 2.0)[f]		
CO_2 (%)	0.08 ± 0.02	0.20 ± 0.06	0.09 ± 0.05	0.20 ± 0.07
CO (ppm)	2.2 ± 0.9	11.5 ± 3.1	2.2 ± 0.9	10.9 ± 2.8
NO_2 (ppm)	0.06 ± 0.04	1.5 ± 0.5	0.06 ± 0.05	1.6 ± 0.5
NO (ppm)	0.08 ± 0.14	8.7 ± 3.6	0.08 ± 0.29	8.3 ± 3.2
SO_2 (ppm)		0.81 ± 0.38	0.01 ± 0.07	0.61 ± 0.29
SO_4^{-2} ($\mu g/m^3$)		29.0 ± 24.9	16.8 ± 17.9	42.3 ± 33.8
Aliphat. aldehydes (ppm)	0.02 ± 0.01	0.12 ± 0.06	0.02 ± 0.01	0.12 ± 0.05
Formaldehyde (ppm)	0.0076 ± 0.0035	0.0383 ± 0.0230	0.0074 ± 0.0041	0.0374 ± 0.0266
Acetaldehyde (ppm)	0.0015 ± 0.0035	0.0387 ± 0.0153	0.0009 ± 0.0025	0.0377 ± 0.014
Acrolein (ppm)	0.0030 ± 0.0033	0.0602 ± 0.0245	0.0062 ± 0.0047	0.0578 ± 0.0205
NH_3 (ppm)	0.52 ± 0.28	0.64 ± 0.71	0.57 ± 0.52	0.48 ± 0.55
NH_4^+ (ppm)		0.027 ± 0.0307	0.0065 ± 0.0143	0.0165 ± 0.0233
THC (ppm)	4.1 ± 1.9	7.5 ± 2.2 (cold)		7.4 ± 2.0 (cold)
PAH ($\mu g/m^3$) Benzo(a)pyrene		13.5 ± 6.8		10.2 ± 6.5
Benzo(a)anthracene		19.6 ± 9.9	3.2 ± 2.2	11.2 ± 5.2
Benzo(k)fluoranthene		5.6 ± 2.3		3.6 ± 2.4
Fluoranthene		139.3 ± 98.1	26.5 ± 11.5	67.5 ± 52.4
Pyrene		123.4 ± 72.2	32.3 ± 15.1	60.0 ± 36.6

[a] All ± are S.D., unless specified otherwise.
[b] < 7μm.
[c] Mass median diameter.
[d] Geometric standard deviation.
[e] Electrical aerosol size analyzer.
[f] Scanning electron microscope.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	National Institute for Occupational Safety and Health							
Reference	Green et al., 1983; Rabovsky et al., 1986				Rabovsky et al., 1984			
Engine type	Caterpillar, 7 L, 4 cylinder, 4-cycle				Caterpillar, 7 L, 4 cylinder, 4-cycle			
Operating mode	8-mode mining cycle, 60% idling				8-mode mining cycle, 60% idling			
Fuel type	No. 2 diesel				No. 2 diesel			
Fuel sulfur	< 0.5%							
Exposure regime	7 h/d, 5 d/week, 12 mo.				7 h/d, 5 d/week, 24 mo.			
Exposure conditions	Control	Exhaust	Coal dust	Exhaust + coal dust	Control	Exhaust	Coal dust	Exhaust + coal dust
Particle conc. (mg/m^3)		2	5	3				
Respirable particles[b] (mg/m^3)		2.01	1.97	2.08		1.9 ± 0.3	2.1 ± 0.4	2.0 ± 0.3
Particle size (μm) MMD[c] (GSD)[d]								
CO_2 (%)	0.08	0.21	0.09	0.20	0.07 ± 0.02	0.16 ± 0.04	0.08 ± 0.04	0.17 ± 0.06
CO (ppm)	2.3	12.7	2.4	11.1	2.0 ± 0.9	10.5 ± 2.3	2.1 ± 0.8	10.3 ± 2.0
NO_2 (ppm)	0.04	1.6	0.04	1.3	0.06 ± 0.04	1.5 ± 0.5	0.07 ± 0.05	1.5 ± 0.05
NO (ppm)	0.07	9.7	0.08	1.3	0.08 ± 0.13	7.8 ± 3.1	0.08 ± 0.28	7.6 ± 2.8
SO_2 (ppm)	0.01	0.83		0.56		0.6 ± 0.4	0.003 ± 0.05	0.5 ± 0.3
SO_4^{-2} ($\mu g/m^3$)								
Aliphatic aldehydes								
Formaldehyde (ppm)								
Acetaldehyde (ppm)								
Acrolein (ppm)								
NH_3 (ppm)	0.63	1.13	0.83	0.54	0.5 ± 0.6	0.6 ± 0.8	0.6 ± 0.7	0.4 ± 0.3
NH_4^+ (ppm)								
THC (ppm)								
PAH ($\mu g/m^3$) Benzo(a)pyrene								
Nitropyrene								

[a] All ± are S.D. unless specified otherwise.
[b] < 7μm.
[c] Mass median diameter
[d] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	General Motors Research Lab					
Reference	Barnhart et al., 1981, 1982; Chaudhari et al., 1980, 1981; Chaudhari and Dutta, 1982; Chen and Vostal, 1981; Dziedzic, 1981; Eskelson et al., 1981; Penney et al., 1981: Misiorowski et al., 1980, 1981; Navarro et al., 1981; Schneider and Felt, 1981; Schreck et al., 1980, 1981; Strom, 1984; Vostal et al., 1981; Wallace et al., 1987; White and Garg, 1981				Gross, 1981	
Engine type	1978 350D Oldsmobile, 5.7 L, 4-cycle				5.7 L	
Operating mode	1350 rpm, 96 N·m				1350 rpm, 96 N·m	
Fuel type	Amoco type 2D				Amoco type 2D	
Fuel sulfur	0.27%				0.27%	
Exposure regime	20 h/d, 5½ d/week, 104 weeks				20 h/d, 5½ d/week, 87 weeks	
Exposure conditions	Control	Exhaust	Exhaust	Exhaust	Control	Exhaust
Particle conc. (mg/m^3)	0.007 ± 0.009	0.258 ± 0.087	0.796 ± 0.228	1.533 ± 0.346	0.007 ± 0.009	1.533 ± 0.346
Particle size (μm) MMD[b] (GSD)		0.19				0.2
CO_2 (%)						
CO (mg/m^3)	2.2 ± 0.6	3.4 ± 0.8	5.3 ± 0.9	7.9 ± 2.1	1.9	7
NO_2 (ppm)						0.5
NO (ppm)						6.7
NO_x (mg/m^3)	0.05	2.1 ± 0.6	5.0 ± 1.2	9.2 ± 1.6	<0.04	7.2
Sulfur (mg/m^3)						1.4
SO_2 (ppm)						
Aliphatic aldehydes						
Formaldehyde (ppm)						
Acrolein (ppm)						
NH_4^+						
THC (ppm)						
PAHs Benzo(a)pyrene						
Nitropyrene						

[a] All ± are S.D., unless specified otherwise.
[b] Mass median diameter.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]				
Facility/Sponsor	Inhalation Toxicology Research Institute			
Reference	Bice et al., 1985; Cheng et al., 1984; Henderson et al., 1983, 1985, 1988; Mauderly et al., 1983, 1984, 1987a, b, 1988; McClellan et al., 1986; Wolf et al., 1987			
Engine type	1980 Oldsmobile V8, 5.7 L			
Operating mode	Federal Test Procedure, urban driving cycle			
Fuel type	Phillips No. 2 diesel			
Fuel sulfur	0.34%			
Exposure regime	7 h/d, 5 d/week, 130 weeks			
Exposure conditions	Control	Exhaust	Exhaust	Exhaust
Particle conc. (mg/m^3)	0.013 ± 0.006	0.353 ± 0.071	3.469 ± 0.447	7.082 ± 0.808
Particle size (μm) MMD[b] (GSD)[c]		0.183 ± 0.04 (4.8 ± 0.28)[d] 0.262 ± 0.06 (4.2 ± 0.24)[e]	0.184 ± 0.02 (5.3 ± 0.64)[d] 0.249 ± 0.03 (4.5 ± 0.54)[e]	0.213 ± 0.06 (4.7 ± 0.94)[d] 0.234 ± 0.06 (4.4 ± 0.88)[e]
CO_2 (%)	0.2005 ± 0.0390	0.2284 ± 0.0371	0.4355 ± 0.0590	0.6643 ± 0.1320
CO (ppm)	1.0 ± 0.7	2.9 ± 1.0	16.5 ± 7.1	29.7 ± 12.9
NO_2 (ppm)	0	0.05 ± 0.09	0.34 ± 0.22	0.68 ± 0.48
NO (ppm)	0	0.7 ± 0.3	5.7 ± 1.5	10.0 ± 2.6
SO_2 (ppm)				
SO_4^{-2} ($\mu g/m^3$)				
Aliphatic aldehydes (ppm)				
Formaldehyde (ppm)				
Acrolein (ppm)				
Ammonia (ppm)	1.1 ± 3.0	1.4 ± 1.3	0.9 ± 0.9	0.7 ± 0.6
Hydrocarbons (ppm)	2.6 ± 0.6	3.8 ± 0.9	8.7 ± 5.2	13.4 ± 8.3
PAHs Benzo(a)pyrene				
Nitropyrene				

[a] All ± are S.D. unless specified otherwise; data for particles through 30 mo.; data for gases from 35th week through 30 mo.
[b] Mass median diameter.
[c] Geometric standard deviation.
[d] Lovelace multiple jet impactor, mass median aerodynamic diameter.
[e] Impactor/parallel flow diffusion battery, mass median diameter.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]								
Facility/Sponsor	Inhalation Toxicology Research Institute							
Reference	Inhalation Toxicology Research Institute - Annual Report, 1980				Mauderly et al., 1981[b]			
Engine type	1980 GM, 5.7 L				1980 GM, 5.7 L			
Operating mode	California 7-mode urban cycle				California 7-mode urban cycle			
Fuel type	Phillips No. 2 diesel				Phillips No. 2 diesel			
Fuel sulfur								
Exposure regime	7 h/d, 5 d/week, 12 weeks				7 h/d, 5 d/week, 19 weeks			
Exposure conditions	Control	Exhaust	Exhaust	Exhaust	Control	Exhaust	Exhaust	Exhaust
Particle conc. (mg/m^3)	0.039 ± 0.020	0.230 ± 0.073	1.030 ± 0.340	4.260 ± 1.110	0.050 ± 0.024	0.210 ± 0.070	1.020 ± 0.350	4.380 ± 1.160
Particle size (μm) MMD[c] (GSD)[d]								
CO_2 (%)				0.2080 ± 0.04				
CO (ppm)	1.1 ± 0.6	1.5 ± 0.6	3.7 ± 1.1	11.5 ± 2.6				
NO_2 (ppm)				0.4 ± 0.4				
NO (ppm)				0.80 ± 0.25				
NO_x (ppm)								
SO_2 (ppm)								
SO_4^{-2} ($\mu g/m^3$)								
O_2 (%)								
Ozone (ppb)				14.6 ± 3.1				
Aliphatic aldehydes								
Formaldehyde (ppm)								
Acrolein (ppm)								
Ammonia	2.8 ± 0.7	3.2 ± 0.8	2.9 ± 0.9	2.5 ± 0.7				
Hydrocarbons (ppm)				4.0 ± 0.8				
HTHC (ppm)								
PAHs Benzo(a)pyrene								
Nitropyrene								

[a] All ± are S.D. unless specified otherwise.
[b] Concentrations of gaseous components reported to be proportional to these in 12-week study.
[c] Mass median diameter.
[d] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	Japan Automobile Research Institute Inc. (Health Effects Research Program - HERP)									
Reference	HERP 1988; Ishinishi et al., 1986; Ishinishi et al., 1989									
Engine type	Light duty, 1.8 L, 4-cylinder, swirl chamber					Heavy duty, 11 L, 6-cylinder, direct injection				
Operating mode	1700 rpm, eddy current dynamometer					1200 rpm, eddy current dynamometer				
Fuel type	Nippon Oil Co JIS No. 1 or 2 diesel					Nippon Oil Co JIS No. 1 or 2 diesel				
Fuel sulfur	0.41%					0.41%				
Exposure regime	16 h/d, 6 d/week, 30 mo.					16 h/d, 6 d/week, 30 mo.				
Exposure conditions	Control	Exhaust	Exhaust	Exhaust	Exhaust	Control	Exhaust	Exhaust	Exhaust	Exhaust
Particle conc. (mg/m^3)	0.003	0.11	0.41	1.08	2.32	0.002	0.46	0.96	1.84	3.72
Particle size (μm) MMD[b] (GSD)[c]				0.19 (2.37-2.71)	0.21-0.22 (2.23-2.93)				0.20-0.23 (2.73-3.07)	0.25-0.28 (2.75-3.18)
CO_2 (%)	0.026	0.050	0.105	0.219	0.418	0.035	0.084	0.140	0.215	0.360
CO (ppm)	0.80	1.23	2.12	3.96	7.10	0.63	2.65	4.85	7.75	12.91
NO_2 (ppm)	0.011	0.08	0.26	0.70	1.41	0.021	0.46	1.02	1.68	3.00
NO (ppm)	0.033	1.16	3.81	9.44	18.93	0.042	5.71	12.11	19.99	34.45
NO_x (ppm)	0.044	1.24	4.06	10.14	20.34	0.061	6.17	13.13	21.67	37.45
SO_2 (ppm)	0.06	0.38	1.06	2.42	4.70	0.06	0.98	1.79	2.82	4.57
SO_4^{-2} ($\mu g/m^3$)	0.41	18.8	62.4	151	315	0.49	62.9	111	198	361
O_2 (%)	20.8	20.8	20.7	20.5	20.3	20.8	20.8	20.7	20.6	20.4
Aliphatic aldehydes										
Formaldehyde (ppm)	0.002	0.01	0.03	0.07	0.13	0.003	0.05	0.11	0.18	0.29
Acrolein (ppm)										
NH_4^+										
LTHC (ppm)	2.17	2.27	2.51	2.87	3.57	3.50	4.27	5.16	5.90	7.62
HTHC (ppm)	2.20	2.44	2.93	3.82	5.49	2.43	4.63	7.15	9.94	15.65
PAHs (ng/m^3) Benzo(a)pyrene					5.3 ± 10.6					7.5 ± 3.2
Benzo(k)fluoranthene					5.4 ± 7.7					6.0 ± 3.0
Benzo(ghi)perylene					2.7 ± 3.9					8.9 ± 2.5
1-Nitropyrene					46.6 ± 44.0					43.4 ± 9.8

[a] All ± are S.D., unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	Japan Automobile Research Institute Inc. (Health Effects Research Program - HERP)				
Reference	HERP, 1988; Ishinishi et al., 1986; Ishinishi et al., 1989				
Engine type	Heavy duty, 11 L, 6-cylinder, direct injection				
Operating mode	1200 rpm, eddy current dynamometer				
Fuel type	Nippon Oil Co JIS No. 1 or 2				
Fuel sulfur	0.41%				
Exposure regime	16 h/d, 6 d/week, 30 mo.				
Exposure conditions	Control	Exhaust, filtered	Exhaust	Exhaust, filtered	Exhaust
Particle conc. (mg/m^3)	0.004	0.005	0.39	0.019	2.99
Particle size (μm) MMD[b] (GSD)[c]					0.31-0.35 (2.58-2.83)
CO_2 (%)	0.068	0.083	0.084	0.391	0.412
CO (ppm)	0.06	2.54	2.50	13.00	12.90
NO_2 (ppm)	0.024	0.42	0.44	3.96	4.95
NO (ppm)	0.040	5.16	5.37	32.81	31.50
NO_x (ppm)	0.062	5.58	5.81	36.76	36.45
SO_2 (ppm)	0.03	0.96	0.98	4.50	4.03
SO_4^{-2} ($\mu g/m^3$)	0.35	1.43	57.7	1.61	358
O_2 (%)	20.8	20.7	20.7	20.4	20.3
Aliphatic aldehydes					
Formaldehyde (ppm)	0.003	0.04	0.04	0.24	0.20
Acrolein (ppm)					
NH_4^+					
LTHC (ppm)	3.62	4.43	4.41	7.79	7.68
HTHC (ppm)	2.38	3.74	4.53	12.68	13.79
PAHs Benzo(a)pyrene					
Nitropyrene					

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]					
Facility/Sponsor	Fraunhofer Institut fur Toxikologie und Aerosolforschung				
Reference	Heinrich et al. 1982		Heinrich et al., 1986; Stober, 1986		
Engine type	2.4 L		1.6 L		
Operating mode	Constant load of 16kW, 2400 rpm		FTP (1972)		
Fuel type	European reference fuel		European reference fuel		
Fuel sulfur	0.36%		0.36%		
Exposure regime	7-8 h/d, 5 d/week, 104 weeks		19 h/d, 6 d/week, 120-140 weeks		
Exposure conditions	Exhaust	Exhaust, filtered	Control	Exhaust	Exhaust, filtered
Particle conc. (mg/m^3)	3.9 ± 0.5			4.24 ± 1.42	
Particle size (μm) MMD[b]	0.1			0.35 ± 0.10	
CO_2 (%)	0.54 ± 0.15	0.52 ± 0.13	0.10 ± 0.01	0.38 ± 0.05	0.35 ± 0.05
CO (ppm)	18.5 ± 4.9	18.0 ± 4.4	0.16 ± 0.27	12.5 ± 2.18	11.1 ± 1.92
NO_2 (ppm)	1.2 ± 1.7	1.0 ± 1.5	-	1.5 ± 0.33	1.2 ± 0.26
NO (ppm)	16.5 ± 5.8	17.2 ± 5.9	-	10.0 ± 2.09	8.7 ± 1.84
NO_x (ppm)	18.6 ± 5.8	19.2 ± 6.1	-	11.4 ± 2.09	9.9 ± 1,80
SO_2 (ppm)	3.1 ± 1.8	2.8 ± 1.7	-	1.12 ± 0.89	1.02 ± 0.62
SO_4^{-2} ($\mu g/m^3$)					
O_2 (vol%)	19.5 ± 0.6	20.0 ± 0.7			
Aliphatic aldehydes					
Formaldehyde (ppm)					
Acrolein (ppm)					
NH_4^+					
THC (ppm)	9.3 ± 4.6	7.9 ± 3.3	3.5 ± 0.29	5.5 ± 0.69	5.2 ± 0.65
CH_4 (ppm)	3.0 ± 2.2	2.6 ± 1.8	2.3 ± 0.17	2.6 ± 0.19	2.4 ± 0.20
PAHs ($\mu g/g$ part.): Benzo(a)pyrene	7.0			3 (13 ng/m^3)	
Benzo(e)pyrene	14.1			- (21 ng/m^3)	
Benz(a)anthracene	9.8				
Fluoranthene	134.6				
Pyrene	65.8				
Benzo(a)fluoranthene	5.4				
Benzo(b)fluoranthene	5.3			- (51 ng/m^3)	
Benzo(ghi)perylene	21.4				
Chrysene	25.7				

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]							
Facility/Sponsor	Fraunhofer Institut fur Toxikologie und Aerosolforschung						
Reference	Heinrich et al., 1979; Meiss et al., 1981						
Engine type	2.4 L						
Operating mode	Constant load of 16 kW, 2400 rpm						
Fuel type	European reference fuel						
Fuel sulfur	0.36%						
Exposure regime	7-8 h/d, 5 d/week, 5 mo.						
Exposure conditions	Control	Exhaust	Exhaust, filtered	Exhaust	Exhaust, filtered	Exhaust	Exhaust, filtered
Particle conc. (mg/m^3)		4		11		17	
Particle size (μm)[b]		0.1		0.1		0.1	
CO_2 (%)	0.1	0.5	0.5	0.9	0.95	1.4	1.6
CO (ppm)	<1	11	11	25	27	42	45
NO_2 (ppm)		0.6	0.5	1.5	1.3	2.6	2.7
NO (ppm)		25	22	43	43	75	68
NO_x (ppm)		26	23	45	44	78	71
SO_2 (ppm)	<1	3	4	8	8	13	12
SO_4^{-2} ($\mu g/m^3$)							
O_2 (vol%)							
Aliphatic aldehydes							
Formaldehyde (ppm)							
Acrolein (ppm)							
NH_4^+							
THC (ppm)	6	8	8	11	12	13	13
CH_4 (ppm)		5	5	5	5	5	5
PAHs Benzo(a)pyrene							
Nitropyrene							

[a] Values estimated from graphically depicted data.
[b] Aerodynamic diameter of the modal peak of the particle mass distribution.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]					
Facility/Sponsor	Southwest Research Institute				
Reference	Kaplan et al., 1983; White et al., 1983				Kaplan et al., 1982
Engine type	5.7 L				5.7 L
Operating mode	Steady state, 1347 rpm, equivalent to constant 40 mph				Steady state, 40 mph
Fuel type	Emissions 2D				
Fuel sulfur	0.23-0.24%				
Exposure regime	20 h/d, 7 d/week, 65 weeks				20 h/d, 7 d/week, 12-13 weeks
Exposure conditions	Control	Exhaust	Exhaust	Exhaust	Exhaust
Particle conc. (mg/m^3)	0.01 ± 0.009	0.242 ± 0.049	0.735 ± 0.084	1.500 ± 0.136	1.500
Particle size (μm)		88-93% < 1.0 79-85% < 0.5	88-94% < 1.0 76-84% < 0.5	91-94% < 1.0 81-85% < 0.5	
CO_2 (%)	0.0649 ± 0.0020	0.0781 ± 0.0028	0.1026 ± 0.0043	0.1355 ± 0.0062	
CO (ppm)	5.81 ± 0.2	6.39 ± 0.3	7.43 ± 0.3	9.40 ± 0.5	
NO_2 (ppm)					
NO (ppm)	0	0.56	1.69	3.42	
NO_x (ppm)	0.05 ± 0.0	0.65 ± 0.1	1.85 ± 0.2	3.73 ± 0.4	
SO_2 (ppm)					
SO_4^{-2} ($\mu g/m^3$)					
O_2 (%)					
Aliphatic aldehydes					
Formaldehyde (ppm)					
Acrolein (ppm)					
NH_4^+					
Hydrocarbons (ppm)	3.43 ± 0.2	3.76 ± 0.3	4.31 ± 0.3	4.99 ± 0.3	
PAHs Benzo(a)pyrene					
Nitropyrene					

[a] All ± are S.D. unless specified otherwise.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	Battelle-Geneva Research Center					Japan Anti-Tuberculosis Association		
Reference	Brightwell et al., 1986; Bernstein et al., 1984					Iwai et al., 1986		
Engine type	1.5 L					2.37 L		
Operating mode	FTP - 1972					Steady state, 1000 rpm		
Fuel type								
Fuel sulfur								
Exposure regime	16 h/d, 5 d/week, 104 weeks					8 h/d, 7 d/week, 96 weeks		
Exposure conditions	Control	Exhaust	Exhaust	Exhaust	Exhaust, filtered	Control	Exhaust, filtered	Exhaust
Particle conc. (mg/m^3)		0.7	2.2	6.6				4.9 ± 1.6
Particle size (μm) MMD[b] (GSD)[c]								
CO_2 (%)				0.46 ± 0.03[e]	0.47 ± 0.03[e]			
CO (ppm)	1 ± 3			32 ± 11	32 ± 11		7.0 ± 1.4[d]	7.0 ± 1.4[d]
NO_2 (ppm)							1.8 ± 1.8[d]	1.8 ± 1.8[d]
NO (ppm)				5.8 ± 2.0[e]	6.0 ± 2.0[e]			
NO_x (ppm)	0.1 ± 0.1			8 ± 1	8 ± 2		30.9 ± 10.9[d]	30.9 ± 10.9[d]
SO_2 (ppm)							13.1 ± 3.6[d]	13.1 ± 3.6[d]
SO_4^{-2} ($\mu g/m^3$)								
O_2 (%)								
Aliphatic aldehydes								
Formaldehyde (ppm)								
Acrolein (ppm)								
NH_4^+								
Hydrocarbons (ppm)				18.9 ± 4.1[e]	18.8 ± 4.1[e]			
PAHs Benzo(a)pyrene								
Nitropyrene								

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.
[d] Samples from dilution tunnel, exposure chamber reported to have approximately the same concentrations.
[e] Data from first year of study (Bernstein et al., 1984).

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]				
Facility/Sponsor	Battelle, Pacific Northwest Laboratory			
Reference	Karagianes et al., 1981			
Engine type	43 bhp, 3 cylinder			
Operating mode	Simulated mining cycle			
Fuel type	Equivalent to VV-F-800 A grade DF-2			
Fuel sulfur	-			
Exposure regime	6 h/d, 5 d/week, 87 weeks			
Exposure conditions	Control	Exhaust	Exhaust + coal dust	
Particle conc. (mg/m^3)	-	8.3 ± 2.0	13.5 ± 4.0	
Resp. particles (mg/m^3)		95% respirable		
Particle size (μm) MMD[b] (GSD)[c]		0.71 (2.3)		
CO_2 (%)				
CO (ppm)		50 ± 3		
NO_2 (ppm)		4-6		
NO (ppm)				
NO_x (ppm)				
SO_2 (ppm)		< 1		
SO_4^{-2} ($\mu g/m^3$)				
O_2 (%)				
Aliphatic aldehydes (ppm)		< 1		
Formaldehyde (ppm)				
Acrolein (ppm)				
Ammonia (ppm)		26-40		
Hydrocarbons (ppm)				
PAHs Benzo(a)pyrene				
Nitropyrene				

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

Facility/Sponsor	University of Pittsburgh			National Board of Occupational Safety and Health - Sweden	Ministry of Supply Chemical Defense Experimental Establishment			
Reference	Battigelli, 1965			Ulfvarson et al., 1987	Pattle et al., 1957			
Engine type	7 hp, four cycle, single cylinder			1980 Volvo, 6 cylinder	0.568 L, single cylinder			
Operating mode				2,500 rpm	1,600 rpm; A - no load; B - load; C - load plus worn injector; D - no load, high fuel-air ratio.			
Fuel type					47 cetane			
Fuel sulfur					0.51%			
Exposure regime	15-60 min			3 h, 40 min	5 h			
Exposure conditions	Dilution A	Dilution B	Dilution C	Exhaust	A	B	C	D
Particle conc. (mg/m^3)				0.6	74	122	53	1,070
Particle size (μm)								
CO_2 (%)	0.1	0.9	1.1					
CO (ppm)	<20	30	55	4.63	560	410	380	1,700
NO_2 (ppm)	1.3	2.8	4.2	2.07	23	51	43	12
NO (ppm)				4.56				
NO_x (ppm)					46	209	174	44
SO_2 (ppm)	0.2	0.5	1					
SO_4^{-2} ($\mu g/m^3$)								
O_2 (%)	20.5	20.0	19.5					
Aliphatic aldehydes	<1.0	<1-2	1-2		16[b]	6.0[b]	6.4[b]	154[b]
Formaldehyde (ppm)	<0.1	<0.1	<0.1	0.04				
Acetaldehyde				0.17				
Acrolein (ppm)	<0.05	<0.05	<0.05					
NH_4^+								
Hydrocarbons (ppm)	<2.0	2.5	3.2					
Benzene (ppm)				0.06				
Toluene (ppm)				0.35				
PAHs ($\mu g/m^3$): Benzo(a)pyrene				640				
Nitropyrene								

[a] All ± are S.D. unless specified otherwise.
[b] As formaldehyde.

APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]								
Facility/Sponsor	U.S. Environmental Protection Agency							
Reference	Gillespie, 1980; Hyde et al., 1980; Malanchuk, 1980; Orthoefer, 1980; Stara et al., 1980							
Engine type	Automobile gasoline engine							
Operating mode	Urban cycle							
Fuel type								
Fuel sulfur								
Exposure regime	16 h/d, 7 d/week, 68 mo.							
Exposure conditions	Control	Non-irradiated gasoline exhaust (R)	Irradiated gasoline exhaust (I)	SO_2 + H_2SO_4	R + SO_2 + H_2SO_4	I + SO_2 + H_2SO_4	Nitrogen oxides	Nitrogen oxides
Particle conc. (mg/m^3)								
Particle size (μm)								
CO_2 (%)								
CO (ppm)	4.9	97.5 ± 10.0	94.5 ± 19.6	-	98.4 ± 13.8	-	-	-
NO_2 (ppm)	0.04	0.05 ± 0.02	0.94 ± 0.36	-	0.05 ± 0.03	0.89 ± 0.36	0.64 ± 0.12	0.15 ± 0.33
NO (ppm)	0.04	1.45 ± 0.42	0.19 ± 0.29	-	1.51 ± 0.44	0.19 ± 0.29	0.25 ± 0.06	1.67 ± 0.21
NO_x (ppm)								
SO_2 (ppm)	0.03	-	-	0.42 ± 0.22	0.48 ± 0.23	0.42 ± 0.21	-	-
H_2SO_4 (ppm)	-	-	-	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	-	-
Oxidants (ppm as O_3)	0.02	-	0.20 ± 0.09	-	-	0.20 ± 0.08	-	-
Aliphatic aldehydes								
Formaldehyde (ppm)								
Acrolein (ppm)								
NH_4^+								
Hydrocarbons (ppm as CH_4)	2.7	27.5 ± 4.4	23.9 ± 6.1	-	27.4 ± 4.3	23.9 ± 6.0	-	-
PAHs Benzo(a)pyrene								
Nitropyrene								

[a] All ± are S.D. unless specified otherwise.

Appendix B

Alternative Model for Diesel Cancer Risk Assessment

B.1. INTRODUCTION

As previously discussed in Chapter 11, the most appropriate method to assess cancer risk of diesel exhaust is to take into account effects of both particles (carbon core) and organics because evidence exists that both agents are involved in carcinogenic process. The reasons for this conclusion are based on the following observations: (1) organics include a variety of polycyclic aromatic hydrocarbons (PAHs) and nitroaromatics, many of which are known to be carcinogenic; (2) the results of recent studies on inert particles and carbon black in rats strongly support the hypothesis that the carbon core of the diesel particle may be the primary component responsible for the induction of lung cancer; (3) PAHs are unlikely responsible for all observed tumors because they account for less than 0.1 μg/mg particulate matter (Tong and Karasek, 1984); and (4) the observation of disproportionate high tumor incidence in high exposure animals coincides with disproportionate increase of cumulative lung burden of diesel particle as exposure concentration increases.

A workshop on Research Needs for Risk Assessment of Inhaled Particulate Matter was organized and sponsored by the U.S. Environmental Protection Agency (EPA) in March, 1992. The purpose of the workshop was to determine the extent of information that can be used for quantitative risk assessment and to discuss mechanisms of particle-induced lung tumors to serve as a guidance for future research needs. Two major, among several other, conclusions that are relevant to quantitative risk assessment were reached by the Workshop:

(1) particle overloading of the lung tissue may induce both initiation (by PAH specific adducts and adducts through oxygen radicals) and cell proliferation steps in tumor formation, and

(2) more research is needed to improve the risk assessment of particle-induced lung cancers.

Although there are not enough data available to construct a biologically based dose-response model, it is desirable to investigate implications of the hypothetical mechanisms proposed by the workshop. The purpose of the alternative modeling presented in this report is to do just that. Briefly, the biological issues and their implications to quantitative risk assessment that we would like to consider are the following.

1. Particles deposited in lung are phagocytized by alveolar macrophages. Because the phagocytizing macrophages in animals from high-dose group may be more likely to be activated to release mediators including reactive oxygen species, cytokines, and growth factors, it is of interest to determine whether or not the available tumor response data are

consistent with the hypothesis that the particle burden affects both initiation and proliferation in carcinogenic process.

2. Organic materials can also induce specific adducts which may contribute to cell initiation. However, given its low content, the contribution of organics to tumor induction may be very small. Can a dose-response model that is consistent to the proposed biological concept be constructed with both organics and particles as dose metrics?

3. If a model that has the above biological interpretation and is consistent with the bioassay data can be constructed, what would be its implications on quantitative risk assessment of diesel exhaust emissions, and how would its results compare with those predicted by the linearized multistage (LMS) model?

B.2. PRELIMINARY CONSIDERATIONS

In order to evaluate the impact of various biological assumptions on diesel risk assessment, it is necessary to construct a mathematical dose-response model that takes into account the biological mechanisms discussed in the EPA workshop. Because an issue of significant importance in diesel risk assessment is the effect of lung overloading on tumor induction, the model should possess the fol/lowing properties.

1. It should depend on both types of dose metrics: organics, and carbon core. It should allow one to account for the contribution of organics and carbon core individually and/or jointly to tumor induction/formation.

2. It should allow for the possibility that model parameters can change with time because of the increasing lung burden during exposure.

3. The cell proliferation and tumor induction/formation should be stochastic in nature; it is not realistic to assume deterministic clonal growth. For instance, it should not be required to assume that all cells divide at the same age.

To accomplish these goals, we assume that a normal cell can be initiated by both organics and carbon core. Denote the initiation rate by μ_1, which is a function of background and diesel-induced rates (as specified below). Because an initiated cell (I-cell) eventually either goes into cell death, or enters the cell cycle (including cells in quiescence, G_0), it is reasonable to assume that the cell lifetime for an I-cell follows certain probability distribution. Under this model, a cell in G_0 phase is equivalent to the case where it has a very long lifetime with certain probability (i.e., in the right-hand tail of the cell lifetime distribution). At the end of an I-cell's lifetime, it either dies (death) with probability β, divides into two daughter cells (birth) with probability α, or divides into one I-cell and one malignant cell (second transition) with probability μ_2; $\alpha + \beta + \mu_2 = 1$. Instead of assuming that a single malignant cell is equivalent to a tumor as in the MVK

model proposed by Moolgavarkar and colleagues (1979, 1981), we assume that a malignant cell has a certain probability to become a tumor; this probability is assumed dose-dependent, thus allowing for an evaluation of dose effect on tumor progression. It should be noted that the proposed model does not exclude the possibility that it may take more than one step (for a normal cell) to become "initiated." The rate of initiation used in the model should be viewed as a net rate which represents several genetic alterations and repairs.

B.3. MATHEMATICAL MODEL AND PARAMETERS ESTIMATION

We shall proceed to construct a dose-response function P(t:d,D), probability of cancer by time (age) t, which depends on both organic, d, and particle (carbon core), D, and incorporates the biological concept discussed previously. Because the model parameters that are not directly observed in laboratory can only be statistically estimated from high concentration cancer bioassay data, the model constructed should not be considered a valid model of diesel-induced carcinogenesis; uncertainty about the low-dose extrapolation still remains. Some discussions about the need for further laboratory measurements will be given later.

The model with the desirable features discussed above falls into one of several classes of stochastic models that have been developed by EPA's Office of Health and Environmental Assessment (OHEA): namely, the stochastic model which was originally proposed by Chen and Farland (1991) and extended into one with time varying parameters by Tan and Chen (1992). This model will be used as the basis for constructing a biologically based dose-response model. A brief mathematical description is presented in Appendix B-2.

The time to event data from Mauderly et al. (1987) are used to estimate model parameters. The data from Mauderly et al. are useful because they contain information on natural mortality and serial sacrifice of animals with or without (malignant) tumors, valuable information for estimating tumor latency. To utilize the information from serial sacrifice in Mauderly et al. an (E-M) algorithm is derived (see Appendix B-1) and used to calculate maximum likelihood estimates of parameters.

B.3.1. Model Parameters and Notations

The following parameters are incorporated in the dose-response model, which includes initiation rate (μ_1), proliferation rate ($\gamma\alpha$), conversion rate ($\gamma\mu_2$), and probability of progression (q). The death rate for the initiated cells is implicitly defined by $\gamma(1 - \mu_2 - \alpha)$. These parameters are all dose dependent.

D: Dose of carbon core, mg/cm^2 of lung epithelial surface; D varies over time

d: Dose of organics, mg/cm^2 of lung epithelial surface

μ_1: Dose-related initiation rate (per cell per day) that depends on μ_0 (background rate), d, and D by $\mu_1 = \mu_0(1 + ad + bD)$; a and b are paramaters to be estimated.

μ_2: Probability of producing a malignant cell at the end of an initiated cell (I-cell) lifetime

α: The probability that an I-cell divides into two daughter cells at the end of its lifetime

q: Probability that a single malignant cell will develop into a malignant tumor

γ: $1/\gamma$ is the mean I-cell lifetime in days; a cell lifetime ends if it either goes into mitosis, or cell death. Note that if one assumes that the probability for a cell to get into mitosis is about the same as cell death then the mean cell lifetime can be conveniently interpreted as time to mitosis (i.e., cell turnover time); thus, shorter cell lifetime implies more frequent cell division. Note that the time to mitosis is a random variable here, not a fixed constant as in the assumption made in the Greenfield et al. (1984) model that has been used extensively by Cohen and Ellwein (1988) to analyze experimental bladder cancer.

N: Number of (normal) target cells

B.3.2. Practical Considerations

By statistical theory alone the E-M algorithm developed in this report provides an elegant procedure which can be used to test hypotheses whether a particular parameter is influenced by organics and carbon core individually or both together. For instance, one could postulate that the parameter γ (reciprocal of which represents mean cell lifetime) is given by $\gamma(d,D_i) = \gamma_0 + \gamma_{11}d + \gamma_{12}D_i$, and then proceed to test a null hypothesis that $\gamma_{11} = 0$, no effect of organics on cell lifetime. This temptation, however, must be resisted because there would be too many parameters that must be estimated if such statistical tests are to be performed. Therefore, rather than performing such a statistical exercise, we proceed with a biologically plausible assumption that parameters q and γ depend only on lung burden of carbon core, C.

The duration of the Mauderly et al. study was about 940 days. To construct a dose-response model with time-dependent lung burden, the time interval (0,940] is divided into five subintervals; each subinterval spans 6 mo except for the last subinterval, which spans from 730 (2 years) to 940 days. Corresponding to an ambient air concentration of diesel emissions in mg/m^3, the deposition-retention model developed by Yu et al. is used to calculate dosimetric (d, D_i), I = 1, 2, ..., 5, where organics dose, d, is not changing with time because it reaches steady state quickly after exposure begins and D_i is the lung burden of carbon core during the ith subinterval.

The assumptions about dose-parameters relationship are given below.

1. The initiation rate associated with a lung burden $\{d, D_i, I = 1, 2, ..., 5\}$ is given by $\mu_1(d,D_i) = \mu_0(1 + a * d + b * D_i)$, for I = 1, 2, ..., 5. This is the only parameter that is assumed to depend on both d and D.

2. Probability of tumor formation from a malignant cell is assumed to be dependent on lung burden D by $q(D_i) = q_0 + q_1 D_i$, I = 1, 2, ..., 5. To simplify calculation, the possibility that q is also dependent on organics d is not considered.

3. The cell lifetime parameter γ is assumed related nonlinearly to lung burden D by $\gamma(D_i) = \gamma_0 + \gamma_1 \text{Log}(1 + D_i)$, I = 1, 2, ..., 5.

To reduce the number of parameters that must be estimated from the Mauderly data, some of the background parameters (μ_0, q_0, and γ_0) for the dose-response model are estimated from the National Toxicology Program (NTP) historical control rate on Fischer-344 rats (reconstructed from Portier et al., 1986). Giving these background parameters, the dose-related parameters are then estimated by the E-M algorithm, which is described in Appendix B-2. Using tumor response data from Mauderly et al. (1987) and the corresponding dosimetric in Table B-1, the resultant parameter estimates for the model are given in Table B-2. To have some appreciation about the implication of the Mauderly et al. (1987) study, the estimated initiation and proliferation (for I-cells) rates for the study are given in Table B-3. Although these values may not represent reality (because they are not actual laboratory measurements), they could be used as a guidance for future research planning. For instance, Table B-3 (along with a discussion about Table B-7) suggests that a slight increase of proliferation rate could cause a drastic increase on tumor incidence, but only if the initiation rate is high enough. This conclusion seems to suggest that although the promotion effect of growth factors is important for tumor induction, the initiation effect of carbon core and/or organics is also essential.

Table B-1. Dosimetric (mg/cm^2 lung surface) use in modeling[a]

Exposure group	d	D_1	D_2	D_3	D_4	D_5
0.35	2.5E-6	6.23E-5	8.75E-5	8.97E-5	9.02E-5	9.02E-5
3.5	3.6E-5	7.54E-4	2.40E-3	3.91E-3	5.25E-3	6.29E-3
7.08	7.3E-5	1.98E-3	5.49E-3	8.56E-3	1.12E-2	1.44E-2

[a]d is organics; D_i, I = 1, 2, ..., 5, are average lung burden of carbon core over five time intervals. These values are calculated by Yu et al. retention model in Appendix C.

Table B-2. Maximum likelihood estimates for model parameters

Parameter[a]	Estimate
μ_0	1.033E-7
a	1.103E+4
b	3.214E+2
μ_2	7.907E-7
q_0	1.035E-1
q_1	5.332E-2
γ_0	1.662E-2
γ_1	2.647
α	5.443E-1
N[b] (given)	8.80E+7

[a]Background parameters μ_0, q_0, and γ_0 are estimated separately from NTP historical control data.
[b]The number of target cells N is assumed to be 10-fold of Type II cells in mice, which is given in Kauffman (1974). It is not essential for N to be given accurately because $N\mu_0$ appears as a single term in the model; the estimated μ_0 will compensate for the under- or over-estimation of N.

Table B-3. Relative magnitude of initiation $\frac{\mu_1(d,D)}{\mu_0}$ and proliferation $(\Gamma[D]/\Gamma_0)$ potential for the exposed versus control groups in Mauderly et al. (1987) study

		Exposed Groups		
	Time Interval	Low	Mid	High
Initiation	1	1.048	1.639	2.442
	2	1.056	2.168	3.570
	3	1.056	2.654	4.556
	4	1.056	3.084	5.405
	5	1.056	3.419	6.433
Proliferation	1	1.004	1.052	1.137
	2	1.006	1.166	1.379
	3	1.006	1.270	1.590
	4	1.006	1.362	1.770
	5	1.006	1.424	1.989

B.4. RESULTS

The dose-response model predicts only probability of tumor occurrence (i.e., tumor incidence) by time t due to an exposure scenario. Because the probability of tumor occurrence is not directly observable (note that when an animal dies with a tumor, it only tells us that a tumor occurred before that time), the model can not be evaluated against the observed data. (Although it is possible to use the dose-response model, with additional assumptions, to calculate tumor mortality, we prefer to evaluate the dose-response model alone because it will be used to predict cancer risk). To evaluate the reasonableness of the model constructed, it is possible only to compare the observed tumor mortality rate (after adjusting for the competing risk) and the predicted tumor incidence rate, with the understanding that observed values are expected to be smaller (this is particularly true at an early stage of tumor development when a tumor is small) than the predicted tumor incidence. Table B-4 appears to bear this out; all of the predicted tumor incidences are either greater than the observed tumor mortality rates, or within the confidence bounds calculated from the observed tumor mortality. The observed tumor mortality rate is calculated by life-table approach. The probability of tumor mortality can only be calculated up to about 900 days because after 900 days tumors are no longer discovered by natural mortality only; in fact, the majority of tumors are discovered by sacrifice.

B.5. RISK PREDICTIONS UNDER VARIOUS EXPOSURE SCENARIOS

For comparison, excess lifetime risks (see Tables B-5 and B-6) due to various exposure scenarios are calculated by the alternative model and the linearized multistage (LMS) model. Both point (maximum likelihood estimate) and 95% upper bound estimates are provided for the alternative model, whereas only upper bound estimate is provided for the LMS model because its linear component (which is notoriously unstable) is estimated to be 0. The 95% upper bound for the alternative model is calculated by the same approach as for the LMS model; (i.e., by increasing parameters a and b until the log-likelihood exceeds a critical value). To extrapolate from animal-based risk estimates to human, two assumptions are made: (1) lung burden in terms of $\mu g/cm^2$ of lung surface is equally potent between animals and humans, and (2) 6 mo of animal life is equivalent to 18 years of human life. The latter assumption is necessary because life-span must be divided into five subintervals to account for different parameter values.

Table B-5 compares predicted risks for humans due to continuous exposure (24 h/day) calculated by alternative and LMS models. It is interesting to see that risk calculations under various exposure concentrations are very similar using the two different models. Table B-6 gives excess risks due to exposure to 2.6 $\mu g/m^3$ of diesel emissions, 16 h/day, 7 days/week; and 15

Table B-4. Comparison of observed tumor mortality rate and predicted probability of cancer occurrence by time (T) when a (malignant) tumor was observed in rats

Exposure (mg/m^3)	Time tumor observed (days)	Observed tumor mortality rate[a] (95% C. I.)		Predicted tumor rate by time (t)
Control	538	0.0051	(0, 0.015)	0.002
	551	0.010	(0, 0.028)	0.003
0.35	710	0.007	(0, 0.022)	0.005
	863	0.025	(0, 0.063)	0.008
3.50	891	0.016	(0, 0.126)	0.039
	895	0.036	(0, 0.052)	0.040
7.08	646	0.006	(0, 0.019)	0.039
	672	0.013	(0, 0.037)	0.046
	701	0.021	(0, 0.041)	0.054
	729	0.027	(0, 0.059)	0.064
	742	0.039	(0.004, 0.075)	0.069
	798	0.052	(0.009, 0.095)	0.097
	810	0.066	(0.016, 0.115)	0.104
	839	0.081	(0.023, 0.138)	0.123
	840	0.096	(0.032, 0.161)	0.123
	847	0.112	(0.041, 0.183)	0.128
	856	0.129	(0.052, 0.207)	0.135
	859	0.146	(0.063, 0.229)	0.137
	883	0.168	(0.077, 0.259)	0.156
	895	0.191	(0.091, 0.291)	0.166

[a]Calculated by the life table procedure. Note that observed values are mortality, which are expected to be smaller than the (predicted tumor) incidence. This expectation is particularly true at early stage of tumor development when a tumor was small.

$\mu g/m^3$, 8 h/day, 5 days/week. The concentration 2.6 $\mu g/m^3$ was reported by EPA's Office of Mobile Sources to be the annual mean exposure of the U.S. population to diesel particulate matter in 1986 and is only slightly higher than the most recent estimate of 2.03 $\mu g/m^3$ in an EPA draft document (Motor Vehicle-Related Air Toxic Study, April, 1993); the concentration 15 $\mu g/m^3$ was reported to be the particulate exposure for workers on urban freeways in an EPA report by Carey (Air Toxics Emissions from Motor Vehicles, 1987, EPA-AA-TSS-PA-86-5).

Table B-5. Comparison of excess risk for humans due to continuous exposure of various concentrations of diesel exhaust emissions under two different models

	Alternative model[a]		
Exposure concentration ($\mu g/m^3$)	MLE	95% u.b.	LMS model[b]
0.01	7.68E-8	1.35E-7	1.71E-7
0.1	8.12E-7	1.41E-6	1.72E-6
1.0 (unit risk)	8.16E-6	1.65E-5	1.74E-5
100	5.58E-4	9.63E-4	1.74E-4
1,000	2.60E-2	4.22E-2	3.33E-2

[a]MLE: maximum likelihood estimate; 95% u.b.: 95% upper bound estimate. These are calculated using the alternative dose-response model.
[b]LMS: calculated by linearized multistage model (slope = 9.04 per mg/cm^2 of lung surface), using carbon core as dosimetric. Only malignant tumors are used in the calculations.

Table B-6. Excess lifetime risk for humans due to exposure to diesel exhaust emissions, under various exposure scenarios

	Alternative model[a]		
Exposure pattern	MLE	95% u.b.	LMS[b]
2.6 $\mu g/m^3$, 16 h/day, 7 days/week Normal person	1.41E-5	2.44E-5	3.00E-5
2.6 $\mu g/m^3$, 16 h/day, 7 days/week 20 pack-year smoker	2.32E-5	3.61E-5	5.38E-5
15 $\mu g/m^3$, 8 h/day, 5 days/week	3.12E-5	5.17E-5	6.18E-5

[a]MLE: maximum likelihood estimate; 95% u.b.: 95% upper bound estimate. These are calculated using the alternative dose-response model.
[b]LMS: calculated by linearized multistage model, using carbon core as dosimetric. Only malignant tumors are used in the calculations.

For the general population exposed to an ambient air concentration of 2.6 $\mu g/m^3$, the risk to normal (i.e., persons with normal respiratory functions) and smokers of 20 pack-years (as defined by Bohning et al., 1982) are provided. According to Bohning et al., the retention half-life for insoluble particle increases from 296 days for persons with normal respiratory function to 519

days for persons with a smoking history of 20 pack-years. This information is used to reduce the alveolar clearance rate in the dosimetric calculations using the same retention model that is also used to calculate dosimetric in Table B-1.

The excess lifetime risks in Tables B-5 and B-6 are calculated by actuarial life-table approach using the survival probability of the NTP control animals provided in Portier et al. (1986). Conceptually, this approach can be viewed as a weighted average of the probability of cancer occurrence over entire lifetime, weighted by survival probability. This approach is more appropriate than the one used previously in the draft report in which probability of cancer occurrence at a preselected time (730 days) is used to represent the lifetime risk; it is more appropriate because tumors here occur very late in life.

B.6. IMPLICATIONS OF THE ALTERNATIVE MODEL

Before proceeding to discuss implications of the alternative model on risk assessment, it should be noted that the parameters used in the model are estimated on the basis of high exposure concentration cancer bioassay data, not on the basis of data from laboratory measurements (e.g., mitotic rates for cells from normal and preneoplastic lesions measured over time), which usually can be obtained over lower range of exposure concentrations. Therefore, uncertainty associated with low-dose extrapolation still remains. For this reason, we will refrain from using the model to evaluate low-dose risk estimations, but rather to evaluate the relative contribution of each biological component (e.g., initiation by organic and carbon core, individually or jointly) in the model to cancer induction.

On the basis of the constructed alternative dose-response model, some specific inferences could be made from Table B-7 by changing parameter values of the original model. Table B-7 provides a comparison of risks calculated with changed parameters, assuming that animals are exposed to 7.08 mg/m^3 of diesel exhaust emissions, 7 h/day, 5 days/week (which is identical to the exposure pattern of the highest exposed group in Mauderly et al., 1987).

The following observations can be made from Table B-7.

1. When there is no diesel induced initiation (Case 2), the risk is 32% of the original model (i.e., the model without changing parameters), in contrast to 42% when exposure concentration is reduced from 7.08 to 1 mg/m^3 (not shown here). Therefore, the role of diesel-induced initiation in cancer induction increases with increasing exposure concentrations. This conclusion is intuitively obvious because spontaneous induction of initiated cells play a bigger role in cancer induction when concentration is lower. A practical implication of this

Table B-7. Effect of changing dose-dependent initiation and promotion parameters (animals are assumed to be exposed to 7.08 mg/m^3 of diesel emissions in air, 7 h/day, 5 days/week for life [i.e., the highest exposure group in Mauderly et al., 1987])

Case number	Parameters changed	Risk at 938 days	Ratio to original model
1	None (original model)	0.2067	1.00
2	a = 0 b = 0	0.0663	0.321
3	a = 0	0.1616	0.782
4	b = 0	0.1165	0.564
5	a = 2.813a[a] b = 0	0.2007	0.971
6	$\gamma_1 = 1.378\gamma_1$[a]	0.3513	1.700
7	$\gamma_1 = 1.756\gamma_1$[a]	0.4537	2.195
8	$\gamma_1 = 0$	0.0319	0.154

[a]a = 2.813a implies that a is increased by 2.813 times of its original value.

observation is that reduction of non-diesel-induced initiation (e.g., by smoking) could have greater proportion of cancer risk reduction when diesel concentration is low than when the concentration is high.

2. Cases 3 and 4 indicate that initiation by either carbon core, or organics contributes significantly to tumor incidence.

3. Case 5, along with observation Number 2 above, suggests that although diesel-induced I-cells play an important role in cancer induction, the role of initiation, however, could be assumed by either organics or carbon core alone by increasing their respective proportions. One implication is that, although existence of I-cells are important for tumor induction, these I-cells could be induced by any agent that initiates (e.g., smoking).

4. Cases 6 to 8 suggest that a small change of proliferation parameter γ could have a disproportionate change of cancer risk. Because this parameter is assumed a function of carbon core dose, lung burden overloading has a significant effect on cancer incidence. In the absence of better information, it is assumed in this report that carbon core continues to have effect at low doses.

These four observations together suggest that while effect of growth factors (which may increase value of γ) by particle overloading is important, the initiation effect of carbon core and/or organics is also essential. Although this conclusion is only tentative because the model

parameters are estimated on the basis of high concentration bioassay data, they do suggest the importance of studying the role of carbon core and organics on initiation and promotion at low- versus high-exposure concentrations. Does the relative initiation potential between organics and carbon core differ at high and low concentrations? Along with the results in Table B-6, it also suggests that a subcohort of workers who were smokers and exposed to high concentrations of diesel exhaust for a long duration would be expected to have higher lung cancer mortality.

It is interesting to observe from Table B-8 that, under the same exposure conditions, risk is greater when exposure begins later in life. This model-based conclusion is due to the fact that older animals have more spontaneously (including non-diesel) induced initiated cells that have potential to be proliferated, converted to malignant cell, and then progressing to cancer. (Note that the above observation would not contradict any observation that might show that younger animals are more sensitive to diesel exposure than older animals if a treatment induces more initiated cells in the younger animals). Assuming that the above model-generated hypothesis is realistic, an important implication is that initiation by nondiesel agents should be considered when assessing risk to humans due to exposure to diesel emissions.

B.7. CONCLUSIONS AND SUMMARY

1. The risk predictions by both alternative and LMS models are comparable over a range of exposure concentrations that is of practical interest. However, this conclusion is valid only under the assumption that the effect of carbon core on each biological component (e.g., initiation) in the model continues to exist at low doses (see further discussions about

Table B-8. Excess cancer risk to rats 200 and 300 days after termination of 6-mo exposure to D = 7.30E-5 mg/cm^2 of organics, and D = 1.89E-3 mg/cm^2 of carbon core[a]

	Days After Exposure Terminated	
Exposure Period (mo)	**200**	**300**
>6	1.48E-3	1.96E-3
6 to 12	4.01E-3	5.25E-3
12 to 18	8.32E-3	1.05E-2

[a]The lung burden is assumed to be zero over unexposed periods. It may not be a realistic assumption because the lung burden is expected to linger over the following periods after exposure terminated; however, the assumed exposure condition serves the purpose better here.

uncertainties below). Based on the Mauderly et al. (1987) study, the risks associated with continuous exposure to 1 $\mu g/m^3$ of diesel emissions calculated by two different models are summarized below:

	Alternative Model		LMS Model
Lung Tumor Data Used	**MLE**	**95% u.b.**	**95% u.b.**
Malignant tumors	8.16E-6	1.65E-5	1.74E-5
Total tumors	N.A.	N.A.	3.44E-5 (taken from Chapter 11)

2. The model suggests that populations with higher expected background rate (e.g., smokers) may be subjected to higher lung cancer risk than the populations with lower background rate. It is noted that U.S. females have about the same background lung cancer rates as the Fischer-344 rats (about 1 to 2%), whereas U.S. males have a background rate of 6%. However, because most of lung cancers are smokers, the risk to nonsmokers (males or females) should be about the same. The use of the unit risk estimate provided in Chapter 11 may somewhat underestimate risk to smokers (or other respiratory-impaired persons) unless adjustment on lung burden is made. Table B-6 provides an example of such adjustment.

B.8. DISCUSSIONS ABOUT UNCERTAINTIES OF RISK ESTIMATES

Although, it is interesting to note that risk estimates by the LMS model are comparable to those calculated by the alternative model, there are uncertainties about low-dose extrapolation by the alternative (as well as by the LMS) model: first, the model parameters are estimated statistically, not measured in the laboratory; and second, the model parameters are estimated on the basis of high-exposure data, the relationship between a parameter and exposure below the exposure range remains unknown, and the dose-parameter relationship used in the model may not be adequate for low-dose extrapolation. For instance, it is assumed that initiation rate is linearly related to doses of carbon core. Such an assumption needs be evaluated. The risk at low doses would be overestimated in this report if the relationship between initiation rate and carbon core is sublinear (concave upward). The sublinear assumption would be reasonable if there is no effect of initiation by carbon core dose (D) at low concentration. On the other hand, the risk would be underestimated if the relationship is supralinear. Therefore, it is important to evaluate

how increase of diesel-exposure concentration affects initiation rate over low-exposure concentrations. Similarly, it is important to know the relationship between dose of carbon core and cell (I-cells in particular) proliferation at low concentration.

Another aspect of uncertainty is the use of lung burdens (organics and carbon core) calculated by mathematical model, rather than actually measured. However, the impact of this uncertainty with regard to the conclusions reached in this report is not expected to be significantly altered even if the model-based dosimetrics are not accurate because the relative patterns of lung burdens between high- and low-exposure concentrations, and between animals and humans should be about the same. Although there is some observed total lung burden, these data are not used because of the following reasons.

1. The observed data are not separated by organics and carbon core.

2. There are no human data—these data are needed to predict risk in humans.

3. The observed data do not go beyond 730 days.

4. The desire is to be consistent with Chapter 11 so that results can be compared.

B.9. DISCUSSIONS ABOUT FUTURE RESEARCH NEEDS

The single most important use of a biologically based dose-response model in the cancer risk assessment is to reduce uncertainty of low-dose extrapolation when the mechanism for tumor response observed at high doses differs drastically from the low doses. However, this report can focus only on the use of the model to guide future research rather than to actually reduce uncertainty of risk estimate because of our inability to obtain biological parameters in the model. If a chemical is known to induce disproportionately larger cell proliferation (in normal, initiated, and/or malignant cells) at high doses than at low doses, then a model that reflects this fact would be useful. With this in mind, our effort should be to identify "components" of carcinogenesis (e.g., increase of mitotic rate) that are disproportionately more affected at high doses than at low doses and to develop models that incorporate those high-dose effects. For the diesel risk assessment, the "components" that require further study include effects of organics and carbon core, individually or jointly, on initiation, proliferation, conversion, and progression steps of carcinogenesis. In order to use biologically based models of carcinogenesis in risk assessments, one needs to know the relationship between parameter values in a model and exposure (or dose). Ideally, some of these parameters, if not all, should be measured directly in the laboratory, or indirectly estimating from neoplastic and preneoplastic lesions (e.g., number of foci, adenomas, and tumors in a lung).

Cell proliferation is an increase in the cell population of different stages: normal, initiated, or malignant cells. Enhanced cell proliferation of normal target cells may itself increase the frequency of mutations, either by inducing error in replication or by converting DNA adducts to mutations before DNA repair can occur. The model implies that tumor incidence is linearly proportional to initiation rate. On the other hand, enhanced cell proliferation of initiated cells could lead to more than linear increase of tumor incidence. Therefore, proliferation of I-cells has a greater impact on tumor incidence than proliferation of normal cells. However, this does not mean that initiation potential of compounds (organics or carbon core) is not important. As discussed previously, it is important to determine the ability of these compounds to initiate at low versus high doses; this has a significant implication for low-dose extrapolation. From the viewpoint of mathematical modeling, cell proliferation is the result of a decrease of cell death rate and/or an increase of mitotic rate, regardless of underlying biological mechanisms. Therefore, it is logical to construct a model (as is done here) with a proliferation component involving cell death and mitosis, and important to obtain data at the cellular level even if biological mechanism at the molecular level is not yet known. If a more precise mechanism is known and the quantitative data are available, then the proliferation component of the model can be improved by incorporating the available biological information. Most of the two-stage models consider a single malignant cell to be equivalent to a tumor. If a compound is known to affect the cell proliferation of tumor cell population, a model that incorporates tumor progression should be used. For the diesel modeling, we assume that particles could enhance the proliferation of malignant cells. This assumption needs to be verified. Another model-generated hypothesis is that persons with higher number of initiated cells are subjected to higher lung cancer risk when exposed to diesel emissions. (A person could have a higher number of initiated cells due to exposure to diesel and/or nondiesel agents, or simply by acquiring more spontaneously induced initiated cells through aging).

In summary, information that is necessary to construct a biologically based dose-response model includes (1) identifying roles that are played by organics and carbon core (individually or jointly) with respect to initiation, proliferation, conversion, and progression, at low versus high doses; (2) quantitative measurements of cellular dynamics (e.g., mitotic rate) for cells at different stages and exposure concentrations; and (3) relationship between parameters and exposure or dose. Because many biological parameters are expected to be age-dependent, they should be measured over different time points. Furthermore, frequency and size of preneoplastic foci, nodules, and tumors could also provide useful information toward improving risk assessment. Some of these data may be obtained by initiation-promotion type of study.

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APPENDIX B-1
E-M ALGORITHM

The E-M algorithm is derived below. It will be used to calculate maximum likelihood estimate of parameters of the alternative model. Data used for the E-M algorithm is taken from Mauderly et al. (1987), which includes time when an animal died (natural mortality or sacrifice) with or without (malignant) tumors. The computer program for the E-M calculations was developed by Mr. Daliang Chang of the Computer Science Corporation under an EPA contract. The theory of E-M algorithm can be found in Dempster et al. (1977).

Assume that the distinct times when animals died by either natural mortality or sacrifice are $t_1 < t_2 < ... < t_m$. The observations can be classified as follows:

$a_{1x}(I)$: observed number of natural deaths without tumor at time t_i in the treatment group x (There are four groups for diesel data [i.e., x = 1, 2, 3, 4.]),

$a_{2x}(I)$: observed number of natural deaths with tumor at time t_i in the treatment group x,

$b_{1x}(I)$: series sacrifice at time t_i without tumor in the treatment group x,

$b_{2x}(I)$: series sacrifice at time t_i with tumors in the treatment group x.

Let T_d represent the time an animal died and T the time a tumor developed.

$\alpha_x(I) = Pr\{T_d = t_i | T_d \geq t_i, T > t_i, x\}$ (conditional probability of death without tumor)

$\beta_x(I|u) = Pr\{T_d = t_i | T_d \geq t_i, T \in (t_{u-1}, t_u], x\}$ (related to deaths with tumors)

Define,

$$A_x(i) = \prod_{j=1}^{i} [1 - \alpha_x(j)],$$

$$B_x(i|u) = \prod_{j=u}^{i} [1 - \beta_x(j|u)].$$

$$S_x(t) = \Pr\{T \geq t | x\} = \exp[-\int_{t_0}^{t} h(x)dx].$$

The function $S_x(t)$ is the probability of tumor free by time t. The exact form of the hazard function h(x) and $S_x(t)$ are given in the next section.

Let

$a_{2x}(I|u)$ = number of natural death at t_i with tumor developed during $(t_{u-1}, t_u]$, in the treatment group x, $u < I$,

$b_{2x}(I|u)$ = number of sacrifice at t_i with tumor developed during $(t_{u-1}, t_u]$, in the treatment group x, $u < I$,

Then

$$a_{2x}(i) = \sum_{u=1}^{i} a_{2x}(i|u), \quad b_{2x}(i) = \sum_{u=1}^{i} b_{2x}(i|u)$$

Let

$$P_x(i|u) = \frac{A_x(u)S_x(t_u)\Delta_x(t_u)B_x(i-1|u)\beta_x(i|u)}{\sum_{j=1}^{i} A_x(j)S_x(t_j)\Delta_x(t_j)B_x(i-1|j)\beta_x(i|j)},$$

and

$$Q_x(i|u) = \frac{A_x(u)S_x(t_u)\Delta_x(t_u)B_x(i|u)}{\sum_{j=1}^{i} A_x(j)S_x(t_j)\Delta_x(t_j)B_x(i|j)},$$

where

$$\Delta_x(t_i) = \frac{S_x(t_{i-1})}{S_x(t_i)} - 1.$$

Given $a_{2x}(x)$, $\{a_{2x}(i|u), u = 1, ..., i\}$, is an $(i - 1)$-dimension multinomial with parameter $\{a_{2x}(i), P_x(i|u), u = 1, ..., i\}$.

Thus, $$E[a_{2x}(i|u)|a_{2x}(i)] = a_{2x}(i)P_x(i|u).$$

Similarly, $\{b_{2x}(i|u), u = 1, ..., i\}$, is an $(i - 1)$-dimension multinormial with parameters $\{b_{2x}(i), Q_x(i|u), u = 1, ..., i\}$, and

$$E[b_{2x}(i|u)|b_{2x}(i)] = b_{2x}(i)Q_x(i|u).$$

It can be shown that the likelihood function is proportional to

$$L = \prod_x \prod_{i=1}^{m} [S_x(t_i)]^{a_{1x}(i)+b_{1x}(i)+m_x(i)} [\Delta_x(t_i)]^{m_x(i)},$$

where

$$m_x(i) = \sum_{u=i}^{m} [a_{2x}(u|i) + b_{2x}(u|i)].$$

Let

$$R_{1x}(i) = \sum_{j=i}^{m} [a_{1x}(j) + b_{1x}(j) + m_x(j)], \text{ and}$$
$$R_{2x}(i|u) = \sum_{j=i}^{m} [a_{2x}(j|u) + b_{2x}(j|u)].$$

Let

$$\Theta = (\mu_1, \mu_2, \gamma_1, ...)$$

be a vector of parameters in function S;

$$\alpha_x = [\alpha_x(1), \alpha_x(2), ..., \alpha_x(m)], \text{ and}$$
$$\beta_x(u) = [\beta_x(1|u), \beta_x(2|u), ..., \beta_x(m|u)]$$

be vectors of parameters related to conditional probabilities of death with and without tumors. These parameters, along with those in Θ_x will be estimated by the E-M algorithm described below.

The M-step:

Given initial values $a_{2x}(i|u)$ and $b_{2x}(i|u)$, estimate

1. $\overline{\alpha_x}(i) = a_{1x}(i)/R_{1x}(i)$ and
2. $\overline{\beta_x}(i|u) = a_{2x}(i|u)/R_{2x}(i|u)$, and
3. obtain $\overline{\theta_x}$ by maximizing the log of L.

The E-Step:

Given the estimated values on $\alpha_x(i)$, $\beta_x(i)$, and Θ_x from the M-step, compute $P_x(i|u)$ and $Q_x(i|u)$, and obtain estimates of $a_{2x}(i|u)$ and $b_{2x}(i|u)$ by

$$\overline{a_{2x}}(i|u) = a_{2x}(i)\overline{P_x}(i|u), \text{ and}$$
$$\overline{b_{2x}}(i|u) = b_{2x}(i)\overline{Q_x}(i|u).$$

With the estimated values of $a_{2x}(i|u)$ and $b_{2x}(i|u)$ available from the E-step, go back to the M-step. Repeat the same process until estimates are stabilized.

APPENDIX B-2
A TUMOR GROWTH MODEL

The tumor growth model with piece-wise constant parameters is taken from Tan and Chen (1992), which is an extension of a stochastic model developed by Chen and Farland (1991). This model has a similar biological motivation as the two-stage model proposed by Greenfield et al. (1984), which has been used by Cohen and Ellwein (1988) to analyze bladder tumors. However, the two models differ from each other with respect to their mathematical formulations; the one adopted in this report is a stochastic model, whereas the other is a deterministic model and does not allow for parameters estimation because the model does not have complete mathematical expression.

Although its most general form will not be used here because of the lack of data, it is worthwhile to note that the stochastic model by Chen and Farland (1991) has two desirable features: (1) it allows for any cell growth distributions (e.g., Gompertz), rather than limiting only to the exponential distribution as in other existing models; and (2) it incorporates the birth and death of tumor cells, rather than assuming that a tumor is born once a single tumor cell occurs, an assumption made by the MVK model (a model proposed by Moolgavkar et al., 1979, 1981). Therefore, if information on cell lifetime distribution, and the progression phase of tumor development is available, a reasonably realistic model can be constructed.

For completeness of the report, a brief description of the model will be presented here. The following notations are needed for the model:

N(t): number of normal (target) cells at time t,

μ_1: initiation rate, and

f(t): the probability density function for the lifetime of an initiated cell (I-cell).

For an I-cell, at the end of its lifetime it either divides (mitosis) or dies (programmed or nonprogrammed death). If it enters into mitosis, it either divides into two I-cells with probability α, or divides into one I-cell and one malignant cell (M-cell) with probability μ_2. Note that, at the end of a cell's lifetime, the probability for the cell to die is $\beta = 1 - \alpha - \mu_2$. A similar setup (i.e., to allow for any cell lifetime distribution) can be made for an M-cell. However, we will confine ourselves to a simpler version assuming that an M-cell lifetime follows an exponential

distribution. Thus, we can simply assume that an M-cell follows a simple birth-death process; it can either divide into two M-cells with a rate α_m or die with a rate β_m.

When parameters are constant over time (ages), the hazard function is given by

$$h(t) = \mu_1\mu_2\int_0^t N(s)m(t - s)\, ds$$

where

$$m(t) = \frac{(y_2 - y_1)^2\exp[A(t)\alpha(y_2 - y_1)]}{\{(1 - y_1) + (y_2 - 1)\exp[A(t)\alpha(y_2 - y_1)]\}^2};$$

where $y_1 < y_2$ are two real roots of $\alpha y^2 - (\alpha + \beta + \mu_2 q)y + \beta = 0$; $\alpha + \beta + \mu_2 = 1$; $q = 1 - \beta_m/\alpha_m$; $A(t) = \int a(x)dx$, where $a(t) = f(t)/[1 - F(t)]$ is the hazard function of the cell lifetime, $F(t)$ is the cumulative function of $f(t)$. Two special cases of interest here are $a(t) = \gamma$, when the exponential distribution is assumed; and $a(t) = \exp(-\gamma t)$, when the Gompertz distribution is assumed.

When exponential distribution (i.e., $a(t) = \gamma$, or $A(t) = \gamma t$) and $q = 1$ are assumed, the model is equivalent to the MVK model. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed (i.e., $A(t) = [1 - \exp(-\gamma t)]/\gamma$).

For the model with time-dependent parameters, assume that the study begins at time t_0. Divide time scale $(t_0, t]$ into k subintervals $L_j = (t_{j-1}, t_j]$, $j = 1, 2, \ldots k-1$ and $L_k = (t_{k-1}, t_k]$ where $t_k = t$ (note that these subintervals may not be the same subintervals defined by deaths or sacrifice before). The parameters that vary over subintervals $(t_{i-1}, t_i]$, $i = 1, 2, \ldots, k$ are μ_{1j}, α_j, β_j, μ_{2j}, N_j, and those parameters related to $f(t)$. The hazard function is given by

$$h(t) = \sum_{j=1}^{k}[\mu_{1j}\mu_{2j}N_j\int_{t_{j-1}}^{t_j} m_j(t_j - s)ds]\prod_{i=j+1}^{k} m_j(t_i - t_{i-1}),$$

where

$$\prod_{i=j+1}^{k} m_j(t_j - t_{j-1}) = 1, \text{ when } j = k$$

and

$$m_j(t) = \frac{(y_{2j} - y_{1j})^2 \exp[A_j(t)\alpha_j(y_{2j} - y_{1j})]}{\{(1 - y_{1j}) + (y_{2j} - 1)\exp[A_j(t)\alpha_j(y_{2j} - y_{1j})]\}^2},$$

where $y_{1j} < y_{2j}$ are two real roots of $\alpha_j y^2 - (\alpha_j + \beta_j + \mu_{2j}q_j)y + \beta_j = 0$; $\alpha_j + \beta_j + \mu_{2j} = 1$; $q_j = 1 - \beta_{mj}/\alpha_{mj}$, $j = 1, 2, ..., k$.

When exponential distribution (i.e., $A_j(t) = \gamma_j t$ and $q_j = 1$ are assumed, the model is equivalent to the MVK model with piece-wise constant parameters. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed (i.e., when $A_j(t) = \{1 - \exp[-\gamma_j t]\}/\gamma_j$).

For the diesel alternative model, the total time is divided into five (i.e., $k = 5$) subintervals. It is shown in Tan and Chen (1992) that, under the assumption of exponential cell lifetime distribution, the tumor free distribution function, $S_x(t)$, can be written as

$$S(t) = \exp\{-\sum_{j=1}^{k}[A_{jj}(t_{j-1}, s_j) + \sum_{i=1}^{j-1} A_{ij}(t_{i-1}, s_j)]\}$$

where $s_j = t_j$ if $j < k$ and $s_j = t$ if $j = k$, and

$$A_{jj}(t_{j-1}, s_j) = 2N_j\mu_{1j}\mu_{2j}\frac{1}{w_I + z_I}\{-(s_j - t_{j-1}) + \frac{2}{\gamma_{Ij}(w_I - z_I)}\log[1 + \frac{w_I - z_I}{2w_I}(e^{w_I\gamma_{Ij}(s_j - t_{j-1})} - 1)]\},$$

$$A_{ij}(t_{i-1},s_j) = 4N_i\mu_{1i}\mu_{2j}[\frac{1}{\gamma_{Ii}(w_I^2 - z_I^2)}]x$$
$$\{\log[\frac{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i+1,j-1}(t_i,t_{j-1})}{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i,j-1}(t_{i-1},t_{j-1})}]$$
$$-\log[\frac{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i+1,j}(t_i,s_j)}{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{ij}(t_{i-1},s_j)}]\},$$

where,

$W_I = [(\alpha + \beta + \mu_2 q)^2 - 4\alpha\beta]^{1/2}$,

$Z_I = \alpha - \beta - \mu_2 q$, and

$\Delta_{ii}(s,t) = \gamma_i(t - s)$ if both s and t are in the same closed subinterval $[t_{i-1}, t_i]$ and

$$\Delta_{ij}(s,t) = \gamma_i(t_i - s) + \sum_{r=i+1}^{j-1} \gamma_r(t_r - t_{r-1})$$

if $s \epsilon L_i$, $t \epsilon L_j$ with $t_i < t_j$

Appendix C

Models for Calculating Lung Burdens

C.1. INTRODUCTION

As discussed in Chapter 4 the lung burden of diesel exhaust particles (DEPs) during exposure is determined by both the amount and site of particle deposition in the lung and, subsequently, by rates of translocation and clearance from the deposition sites. Mathematical models have often been used to complement experimental studies in estimating the lung burdens of inhaled particles in different species under different exposure conditions. This section presents a mathematical model that simulates the deposition and clearance of DEPs in the lungs of rats and humans.

Diesel particles are aggregates formed from primary spheres of 15 to 30 nm in diameter. The aggregates are irregularly shaped and range in size from a few molecular diameters to tens of microns. The mass median aerodynamic diameter (MMAD) of the aggregates is approximately 0.2 μm. The primary sphere consists of a carbonaceous core (soot) on which numerous kinds of organic compounds are adsorbed. The organics normally account for 10 to 30% of the particle mass. However, the exact size distribution of DEPs and the specific composition of the adsorbed organics depend upon many factors, including engine design, fuels used, engine operating conditions, and the thermodynamic process of exhaust. The physical and chemical characteristics of DEPs have been reviewed extensively by Amann and Siegla (1982) and Schuetzle (1983).

Four mechanisms deposit diesel particles within the respiratory tract during exposure: impaction, sedimentation, interception, and diffusion. The contribution from each mechanism to deposition, however, depends upon lung structure and size, the breathing condition of the subject, and particle size distribution. Under normal breathing conditions, diffusion is found to be the most dominant mechanism. The other three mechanisms play only a minor role.

Once DEPs are deposited in the respiratory tract, both the carbonaceous cores and the adsorbed organics of the particles will be removed from the deposition sites as described in Chapter 4. There are two mechanisms which facilitate this removal: (a) mechanical clearance, provided by mucocilliary transport in the ciliated conducting airways as well as macrophage phagocytosis and migration in the nonciliated airways, and (b) clearance by dissolution. Since the carbonaceous soot of DEPs is insoluble, it is removed from the lung primarily by mechanical clearance, whereas the adsorbed organics are removed principally by dissolution.

C.2. PARTICLE MODEL

To develop a mathematical model which simulates the deposition and clearance of DEPs in the lung, an appropriate particle model characterizing a diesel particle must first be introduced. For the deposition study, we employed an equivalent sphere model for the diesel particle developed by Yu and Xu (1987) to simulate the dynamics and deposition of DEPs in the

respiratory tract by various deposition mechanisms. For the clearance study, we assume that a diesel particle is composed of three different material components according to their characteristic clearance rates: (1) a carbonaceous core of approximately 80% of the particle mass, (2) absorbed organics of about 10% of particle mass, and that are slowly cleared from the lung (3) adsorbed organics quickly cleared from the lung accounting for the remaining 10% of particle mass. The presence of two discrete organic phases in the particle model is suggested by observations that the removal of particle-associated organics from the lung exhibits a biphasic clearance curve (Sun et al., 1984; Bond et al., 1986) as discussed in Chapter 4. This curve represents two major kinetic clearance phenomena: a fast phase organic washout with a half-time of a few hours and a slow phase with a half-time that is a few hundred times longer. The detailed components involved in each phase of the clearance are not known. It is possible that the fast phase consists of organics which are leached out primarily by diffusion mechanisms while the slow phase might include any or all of the following components: (a) organics which are "loosened" before they are released, (b) organics which have become intercalated in the carbon core and where release is thus impeded, (c) organics which are associated for longer periods of time due to hydrophobic interaction with other organic phase materials, (d) organics which have been ingested by macrophages and as a result effectively remain in the lung for a longer period of time due to metabolism by the macrophage; metabolites formed may interact with other cellular components, and (e) organics which have directly acted on cellular components such as the formation of covalent bonds with DNA and other biological macromolecules to form adducts.

The above distinction of the organic components is largely mechanistic and it does not specifically imply the actual component nature of the organics adsorbed on the carbonaceous core; however, the distinction is made to account for the biphasic clearance of DEPs. However, this distinction is necessary in appreciating the dual phase nature of DEPs. For aerosols made of pure organics such as benzo(*a*)pyrene (BaP) and nitropyrene (NP) in the same size range of DEPs, Sun et al. (1984) and Bond et al. (1986) observed a nearly monophasic clearance curve. This might be explained by the absence of intercalative phenomena (a) and of hydrophobic interaction imposed by a heterogeneous mixture of organics (b). The measurement of a pure organic might also neglect that quantity which has become intracellular (c) or covalently bound (d).

C.3. COMPARTMENTAL LUNG MODEL

To study the transport and removal of DEPs from the lungs, we used a compartmental model consisting of four anatomical compartments: the nasopharyngeal or head (H), tracheobronchial (T), alveolar (A), and lung associated lymph node (L) compartments as shown

in Figure C-1. In addition, we used two outside compartments B and G representing, respectively, the blood and gastrointestinal (GI) tract. The alveolar compartment in the model is obviously the most important compartment for long-term retention studies. However, for short-term consideration, retentions in other lung compartments may also be significant. The presence of these lung compartments and the two outside compartments in the model therefore provides a complete description of all clearance processes involved.

In Figure C-1, $r_H^{(i)}$ $r_T^{(i)}$ and $r_A^{(i)}$ are, respectively, the mass deposition rates of DEP material component i (i=1 [core], 2 [slowly cleared organics], and 3 [rapidly cleared organics]) in the head, tracheobronchial and alveolar compartments; and $\lambda_{XY}^{(i)}$ represents the transport rate of material component i from any compartment X to any compartment Y. Let the mass fraction of material component i of a diesel particle be fi. Then

$$r_H^{(i)} = f_i \, r_H \quad , \tag{C-1}$$

$$r_T^{(i)} = f_i \, r_T \quad , \tag{C-2}$$

$$r_A^{(i)} = f_i \, r_A \quad , \tag{C-3}$$

where r_H, r_T, and r_A are, respectively, the total mass deposition rates of DEPs in the H, T, and A compartments, determined from the equations:

$$r_H = c(TV)(RF)(DF)_H \quad , \tag{C-4}$$

$$r_T = c(TV)(RF)(DF)_T \quad , \tag{C-5}$$

$$r_A = c(TV)(RF)(DF)_A \quad . \tag{C-6}$$

In equations C-4 to C-6, c is the mass concentration of DEPs in the air, TV is the tidal volume, RF is the respiratory frequency, and $(DF)_H$, $(DF)_T$, and $(DF)_A$ are, respectively, the deposition fractions of DEPs in the H, T, and A compartments over a respiratory cycle. The values of $(DF)_H$, $(DF)_T$, and $(DF)_A$ which vary with the particle size, breathing conditions and lung architecture were determined from our deposition model (Yu and Xu, 1987).

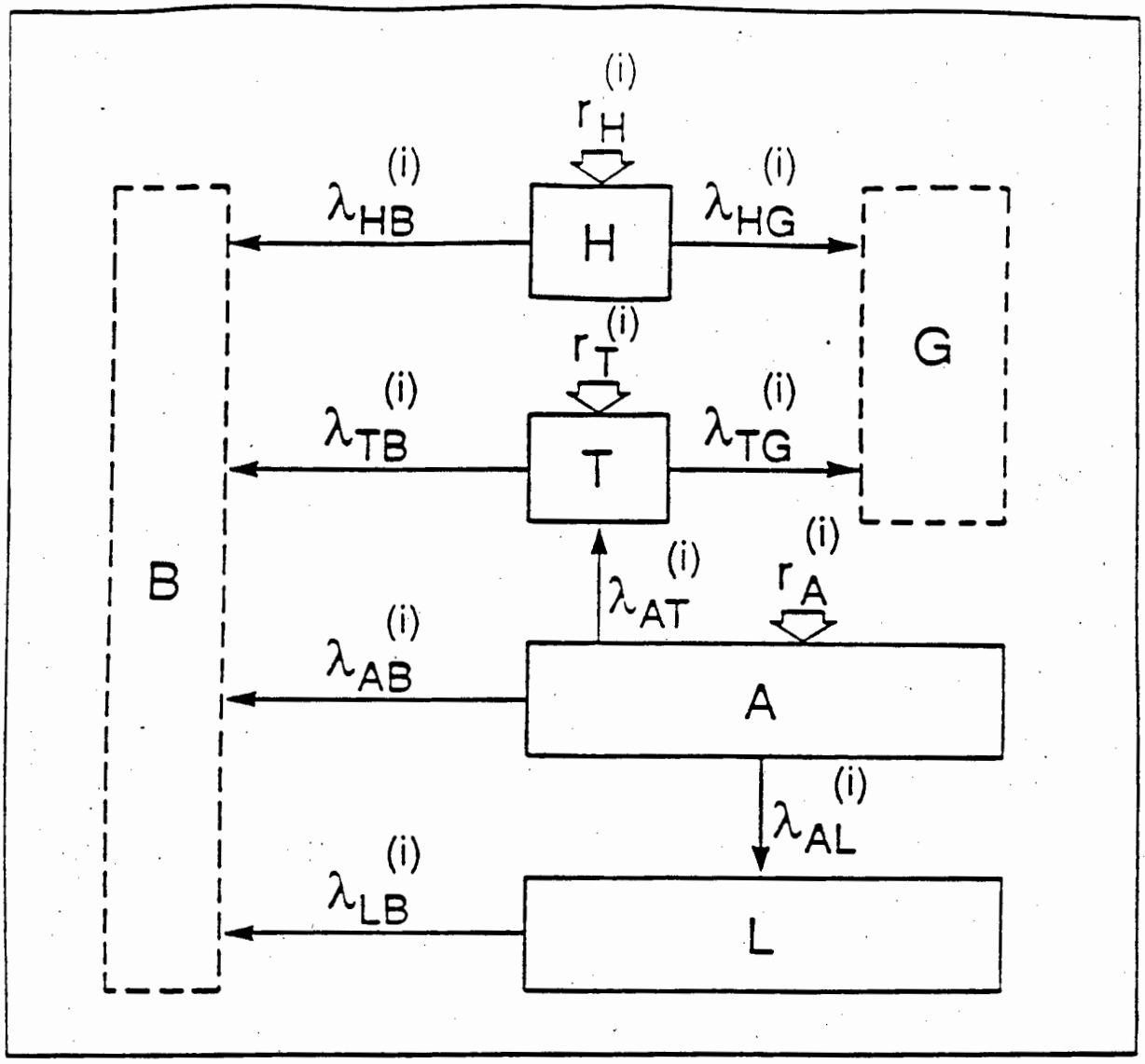

Figure C-1. Compartmental model of DEP retention.

The differential equations for $m_{XY}^{(i)}$, the mass of material component i in compartment X, as a function of exposure time t can be written as

Head (H)

$$\frac{dm_H^{(i)}}{dt} = r_H^{(i)} - \lambda_{HG}^{(i)} m_H^{(i)} - \lambda_{HB}^{(i)} m_H^{(i)} \quad , \qquad \text{(C-7)}$$

Tracheobronchial (T)

$$\frac{dm_T^{(i)}}{dt} = r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{TG}^{(i)} m_T^{(i)} - \lambda_{TB}^{(i)} m_T^{(i)} \quad , \tag{C-8}$$

Alveolar (A)

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{AB}^{(i)} m_A^{(i)} \quad , \tag{C-9}$$

Lymph Nodes (L)

$$\frac{dm_L^{(i)}}{dt} = \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{LB}^{(i)} m_L^{(i)} \quad . \tag{C-10}$$

Equation C-9 may also be written as

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_A^{(i)} m_A^{(i)} \quad , \tag{C-11}$$

where

$$\lambda_A^{(i)} = \lambda_{AT}^{(i)} + \lambda_{AL}^{(i)} + \lambda_{AB}^{(i)} \quad . \tag{C-12}$$

is the total clearance rate of material component i from the alveolar compartment. In equations C-7 to C-10, we have assumed vanishing material concentration in the blood compartment to calculate diffusion transport.

The total mass of the particle-associated organics in compartment X is the sum of $m_X^{(2)}$ and $m_X^{(3)}$the total mass of DEPs in compartment X is equal to

$$m_X = m_X^{(1)} + m_X^{(2)} + m_X^{(3)} \tag{C-13}$$

The lung burdens of diesel soot (core) and organics are defined, respectively, as

$$m_{Lung}^{(1)} = m_T^{(1)} + m_A^{(1)} \quad , \tag{C-14}$$

and

$$m_{Lung}^{(2)+(3)} = m_T^{(2)} + m_A^{(2)} + m_T^{(3)} + m_A^{(3)} \quad . \tag{C-15}$$

Because the clearance of diesel soot from compartment T is much faster than from compartment A, $m_T^{(1)} < m_A^{(1)}$ a short time after exposure, equation C-14 leads to

$$m_{Lung}^{(1)} \cong m_A^{(1)} \quad . \tag{C-16}$$

Solution to equations C-7 to C-10 can be obtained once all the transport rates $\lambda_{XY}^{(i)}$ are known. When $\lambda_{XY}^{(i)}$ are constant, which is the case of linear kinetics, equations C-7 to C-10 will have a solution that increases with time at the beginning of exposure but eventually saturates and reaches a steady-state value. This is the classical retention model developed by the International Commission of Radiological Protection (ICRP, 1979). However, as discussed in Chapter 4, data have shown that when rats are exposed to DEPs at high concentration for a prolonged period, the diesel soot accumulates in various peribronchial and subpleural regions in the lung and the long-termed clearance is impaired. This is the so-called overload effect, observed also for other insoluble particles. The overload effect cannot be predicted by the classical ICRP model. Soderholm (1981) and Strom et al. (1987, 1988) have proposed a model to simulate this effect by adding a separate sequestrum compartment in the alveolar region. In the present approach, a single compartment for the alveolar region of the lung is used and the overload effect is accounted for by a set of variable transport rates $\lambda_{AT}^{(i)}$, $\lambda_{AL}^{(i)}$, and $\lambda_{A}^{(i)}$ which are functions of m_A. The transport rates $\lambda_{A}^{(i)}$ and $\lambda_{AL}^{(i)}$ in equations C-7 to C-10 can be determined directly from experimental data on lung and lymph node burdens, and $\lambda_{AT}^{(i)}$ and $\lambda_{AB}^{(i)}$ from equation C-12.

C.4. SOLUTIONS TO KINETIC EQUATIONS

Equation C-11 is a nonlinear differential equation of $m_A^{(i)}$ with known function of $\lambda_A^{(i)}$. For diesel soot, this equation becomes

$$\frac{dm_A^{(1)}}{dt} = r_A^{(1)} - \lambda_A^{(1)}(m_A) m_A^{(1)} \quad . \tag{C-17}$$

Because clearance of the particle-associated organics is much faster than diesel soot, $m_A^{(2)}$ and $m_A^{(3)}$ constitute only a very small fraction of the total particle mass (less than one percent) after a long exposure and we may consider $\lambda_A^{(1)}$ as a function of $m_A^{(1)}$ alone. Equation C-17 is then reduced to a differential equation with $m_A^{(1)}$ the only dependent variable.

The general solution to equation C-17 for constant $r_A^{(1)}$ at any time, t, can be obtained by the separation of variables to give

$$\int_0^{m_A^{(1)}} \frac{dm_A^{(1)}}{r_A^{(1)} - \lambda_A^{(1)} m_A^{(1)}} = t \quad . \tag{C-18}$$

If $r_A^{(1)}$ is an arbitrary function of t, equation C-17 needs to be solved numerically such as by a Runge-Kutta method. Once $m_A^{(1)}$ is found, the other kinetic equations C-7 to C-10 for both diesel soot and the particle-associated organics can be solved readily, since they are linear equations. The solutions to these equations for constant $r_H^{(i)}$, $r_T^{(i)}$, and $r_A^{(i)}$ are given below:

Head (H)

$$where \quad \lambda_H^{(i)} = \lambda_{HG}^{(i)} + \lambda_{HB}^{(i)} \tag{C-20}$$

Tracheobronchial (T)

$$^{i)} = \exp(-\lambda_T^{(i)} t) \int_0^t (r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)}) \exp(\lambda_{AT}^{(i)} t)\, dt + m \tag{C-21}$$

$$where \quad \lambda_T^{(i)} = \lambda_{TG}^{(i)} + \lambda_{TB}^{(i)} \tag{C-22}$$

Lymph Nodes (L)

$$m_L^{(i)} = \exp(-\lambda_{LB}^{(i)} t) \int_0^t \lambda_{AL}^{(i)} m_A^{(i)} \exp(\lambda_{LB}^{(i)})\, dt + m_{L0}^{(i)}) \tag{C-23}$$

In equations C-19 to C-23, $m_{XO}^{(i)}$ represents the value of $m_X^{(i)}$ at t = 0.

In the sections to follow, the methods of determining $r_H^{(i)}$, $r_T^{(i)}$ and $r_A^{(i)}$ or $(DF)_H$, $(DF)_T$, and $(DF_A$ $r_H^{(DF)}$, $r_T^{(DF)}$ and $r_A^{(DF)}$ as well as the values of $\lambda_{XY}^{(i)}$ in the compartmental lung model are presented.

C.5. DETERMINATION OF DEPOSITION FRACTIONS

The mathematical models for determining the deposition fractions of DEPs in various regions of the respiratory tract have been developed by Yu and Xu (1986, 1987) and are adopted in this report. Yu and Xu consider DEPs as a polydisperse aerosol with a specified mass median aerodynamic diameter (MMAD) and geometrical standard deviation σ_g. Each diesel particle is represented by a cluster-shaped aggregate within a spherical envelope of diameter d_e. The envelope diameter d_e is related to the aerodynamic diameter of the particle by the relation

$$\frac{d_e}{d_a} = \phi^{-1/2}\left(\frac{C_a}{C_e}\right)^{1/2)}\left(\frac{\zeta}{\zeta_o}\right)^{1/2} \tag{C-24}$$

where ζ is the bulk density of the particle in g/cm^3, ζ_0 = 1 g/cm^3; ϕ is the packing density, which is the ratio of the space actually occupied by primary particles in the envelope to the overall envelope volume; and C_x is the slip factor given by the expression:

$$C_x = 1 + 2\frac{\lambda}{d_x}\left[1.257 + 0.4 \exp -\left(\frac{0.55 d_x}{\lambda}\right)\right] \tag{C-25}$$

in which $\lambda \cong 8 \times 10^{-6}$cm^3 is the mean free path of air molecules at standard conditions. In the diesel particle model of Yu and Xu (1986), ζ has a value of 1.5 g/cm^3 and a ϕ value of 0.3 is

chosen based upon the best experimental estimates. As a result, Equation C-24 gives d_e/d_a = 1.35. In determining the deposition fraction of DEPs, d_e is used for diffusion and interception according to the particle model.

C.5.1. Determination of $(DF)_H$

Particle deposition in the naso- or oro-pharyngeal region is referred to as head or extrathoracic deposition. The amount of particles that enters the lung depends upon the breathing mode. Normally, more particles are collected via the nasal route than the oral route because of the nasal hairs and the more complex air passages of the nose. Since the residence time of diesel particles in the head region during inhalation is very small (about 0.1 s for human adults at normal breathing), diffusional deposition is insignificant and the major deposition mechanism is impaction. The following empirical formulas derived by Yu et al. (1981) for human adults are adopted for deposition prediction of DEPs:

For mouth breathing:

$$(DF)_{H,\,in} = 0, \qquad for\ d_a^2 \le 3000 \tag{C-26}$$

$$(DF)_{H,\,in} = -1.117 + 0.324\ \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \tag{C-27}$$

$$(DF)_{H,\,ex} = 0, \tag{C-28}$$

and for nose breathing:

$$(DF)_{H,\,in} = -0.014 + 0.023\ \log(d_a^2 Q),\ for\ d_a^2 Q \le 337 \tag{C-29}$$

$$(DF)_{H,\,in} = -0.959 + 0.397\ \log(d_a^2 Q),\ for\ d_a^2 Q > 337 \tag{C-30}$$

$$(DF)_{H,\,ex} = 0.003 + 0.033\ \log(d_a^2 Q),\ for\ d_a^2 Q \le 215 \tag{C-31}$$

$$(DF)_{H,\,ex} = -0.851 + 0.399\ \log(d_a^2 Q),\ for\ d_a^2 Q > 215 \tag{C-32}$$

where $(DF)_H$ is the deposition efficiency in the head, the subscripts in and ex denote inspiration and expiration, respectively, d_a is the particle aerodynamic diameter in μm, and Q is the air flowrate in cm^3/sec.

Formulas to calculate deposition of diesel particles in the head region of children are derived from those for adults using the theory of similarity, which assumes that the air passage in the head region is geometrically similar for all ages and that the deposition process is characterized by the Stokes number of the particle. Thus, the set of empirical equations from C-26 through C-32 are transformed into the following form:

For mouth breathing:

$$(DF)_{H,\,in} = 0, \qquad for\ d_a^2 Q \le 3000 \tag{C-33}$$

$$(DF)_{H,\,in} = -1.117 + 0.972\ logK + 0.324\ \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \tag{C-34}$$

$$(DF)_{H,\,ex} = 0. \tag{C-35}$$

and for nose breathing:

$$(DF)_{H,\,in} = -0.014 + 0.690\ \log K + 0.023\ \log(d_a^2 Q),\ for\ d_a^2 Q \le 337 \tag{C-36}$$

$$(DF)_{H,\,in} = -0.959 + 1.191\ \log K + 0.397\ \log (d_a^2 Q),\ \text{for}\ d_a^2 Q > 337 \tag{C-37}$$

$$(DF)_{H,\,ex} = 0.003 + 0.099\ \log K + 0.033\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q \le 215 \tag{C-38}$$

$$(DF)_{H,\,ex} = 0.851 + 1.197\ \log K + 0.399\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q > 215 \tag{C-39}$$

where K is the ratio of the linear dimension of the air passages in the head region of adults to that of children, which is assumed to be the same as the ratio of adult/child tracheal diameters.

For rats, the following empirical equations are used for deposition prediction of DEPs in the nose:

$$(DF)_{H,\,in} = (DF)_{H,\,ex} = 0.046 + 0.009\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q \le 13.33 \tag{C-40}$$

C.4.2. Determination of $(DF)_T$ and $(DF)_A$

The deposition model adopted for DEPs is the one previously developed for monodisperse and (Yu, 1978) and polydisperse spherical aerosols (Diu and Yu, 1983). In the model, the branching airways are viewed as a chamber model shaped like a trumpet (Figure C-2). The cross-sectional area of the chamber varies with airway depth, x, measured from the beginning of the trachea. At the last portion of the trumpet, additional cross-sectional area is present to account for the alveolar volume per unit length of the airways.

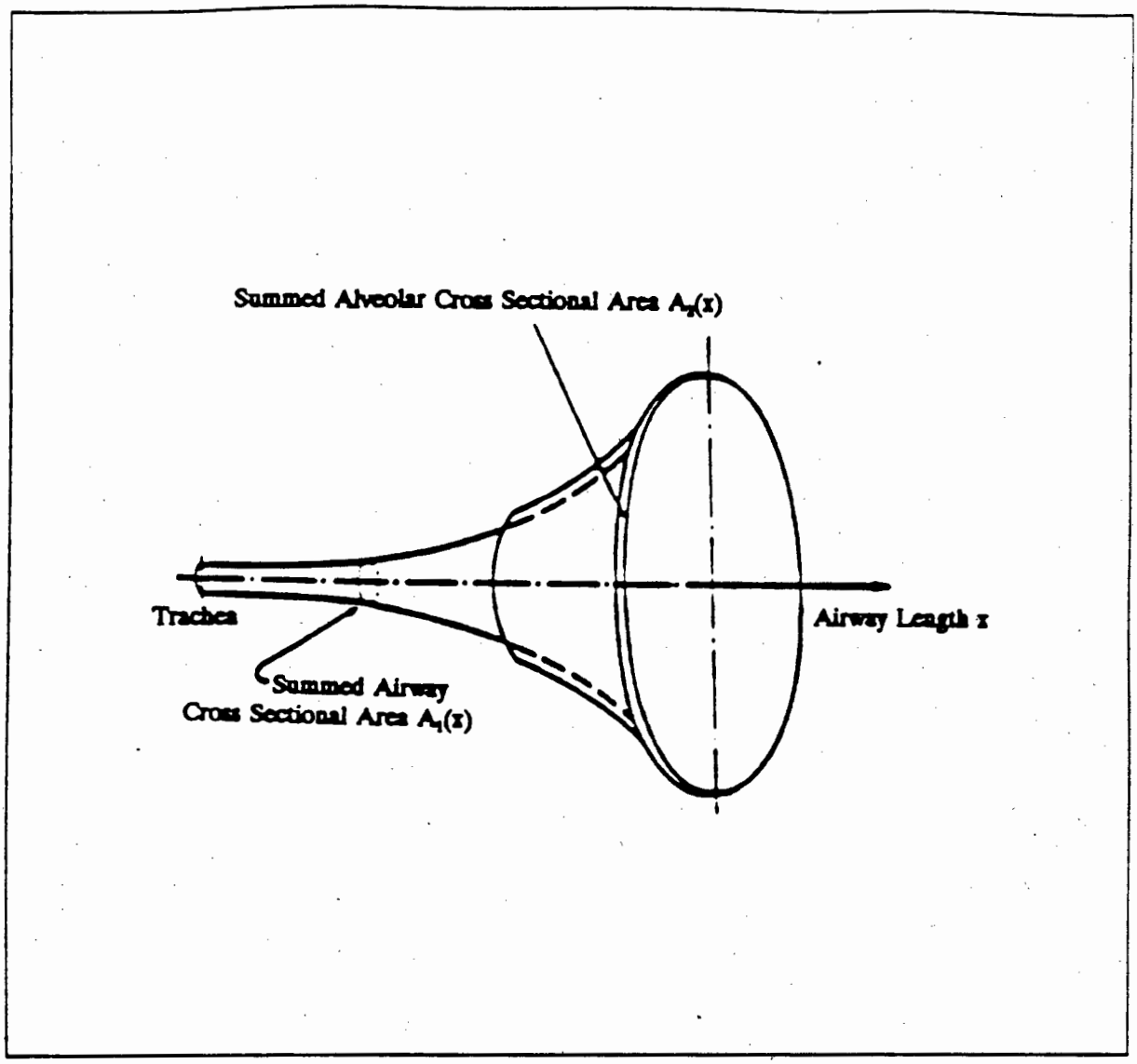

Figure C-2. Trumpet model of lung airways.

Inhaled diesel particles that escape capture in the head during inspiration will enter the trachea and subsequently the bronchial airways (compartment T) and alveolar spaces (compartment A).

Assuming that the airways expand and contract uniformly during breathing, the equation for the conservation of particles takes the form:

$$\beta(A_1 + A_2)\frac{\partial c}{\partial x} + Q\frac{\partial c}{\partial x} = -Qc\eta \qquad \text{(C-42)}$$

where c is the mean particle concentration at a given x and time t; A_1 and A_2 are, respectively, the summed cross-sectional area (or volume per unit length) of the airways and alveoli at rest; η is the particle uptake efficiency per unit length of the airway; β is an expansion factor, given by:

$$\beta = 1 + \frac{V_t}{V_l} \tag{C-43}$$

and Q is the air flow rate, varying with x and t according to the relation

$$\frac{Q}{Q_o} = 1 - \frac{V_x}{V_l} \tag{C-44}$$

where Q_0 is the air flow rate at x = 0. In Equations C-43 and C-44, V_t is the volume of new air in the lungs and V_x and V_ℓ are, respectively, the accumulated airway volume from x = 0 to x, and total airway volume at rest.

Equation C-42 is solved using the method of characteristics with appropriate initial and boundary conditions. The amount of particles deposited between location x_1 and x_2 from time t_1 to t_2 can then be found from the expression

$$DF = \int_{t_1}^{t_2} \int_{x_1}^{x_2} Qc\eta dx dt \tag{C-45}$$

For diesel particles, η is the sum of those due to the individual deposition mechanisms described above, i.e.,

$$\eta = \eta_I + \eta_S + \eta_p + \eta_D \tag{C-46}$$

where η_I, η_S, η_P, and $_D$ are, respectively, the deposition efficiencies per unit length of the airway due to impaction, sedimentation, interception, and diffusion. On the basis of the particle model described above, the expressions for η_I, η_S, η_P, and η_D are obtained in the following form:

$$\eta_I = \frac{0.768}{L}(St)\theta. \tag{C-47}$$

$$\eta_S = \frac{2}{\pi L}[2\epsilon\sqrt{1 - \epsilon^{(2/3)}} - \epsilon^{1/3}\sqrt{1 - \epsilon^{2/3}} + \sin^{-1}\epsilon^{1/3}] \tag{C-48}$$

$$\eta_P = \frac{4}{3\pi L}(\Gamma - \frac{\Gamma^3}{32}) \tag{C-49}$$

$$\eta_D = \frac{1}{L}[1 - 0.819\exp(-14.63\Delta) - 0.0976\ \exp(-89.22\Delta) - 0.0325\ \exp(-228\Delta) - 0.0509\ \exp(-125\Delta^{2/3})] \quad \text{(C-50)}$$

for Reynolds numbers of the flow smaller than 2000, and

$$\eta_D = \frac{4}{L}\Delta^{1/2}\ (1 - 0.444\Delta^{1/2}) \quad \text{(C-51)}$$

for Reynolds numbers greater than or equal to 2000, where $ST=d_a^2u/(18\mu R)$ is the particle Stokes number, $\theta = L/(8R)$, $\epsilon = 3\mu u_s L/(32uR)$, $\Gamma = d_e/R$, and $\Delta = DL/(4R^2u)$. In the above definitions u is the air velocity in the airway; μ is the air viscosity; L and R are, respectively, the length and radius of the airway; $u_s = C_a d_a^2/(18\mu)$ is the particle settling velocity; and $D = C_e kT/(3\pi\mu d_e)$ is the diffusion coefficient with k denoting the Boltzmann constant and T the absolute temperature. In the deposition model, it is also assumed that η_I and $\eta_P = 0$ for expiration, while η_D and η_S have the same expressions for both inspiration and expiration.

During the pause, only diffusion and sedimentation are present. The combined deposition efficiency in the airway, E, is equal to:

$$E = 1 - (1 - E_S)\ (1 - E_D)\ . \quad \text{(C-52)}$$

where E_D and E_S are, respectively, the deposition efficiencies due to the individual mechanisms of diffusion and sedimentation over the pause period. The expression for E_D and E_s are given by

$$1 - \sum_{i=1}^{3} \frac{4}{\alpha_i} \exp(-\alpha_i^2\tau_D)(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2}) \exp\left[- \frac{4\tau_D^{1/2}}{\pi^{1/2}(1 - \sum_{i=}^{3}} \right. \quad \text{(C-53)}$$

where $\tau_D = D\tau/R^2$ in which τ is the pause time and α_1, α_2, and α_3 are the first three roots of the equation:

$$J_o(\alpha) = 0\ . \quad \text{(C-54)}$$

in which J_o is the Bessel function of the zeroth order, and:

$$E_S = 1.1094\tau_S - 0.1604\tau_S^2, \textit{ for } 0 < \tau_S \leq 1. \quad \text{(C-55)}$$

and

$$E_S = 1 - 0.0069\tau_S^{-1} - 0.0859\tau_S^{-2} - 0.0582\tau_S^{-3}, \textit{ for } \tau_S > 1, \quad \text{(C-56)}$$

where $\tau_S = u_S\tau/2R$.

The values of $(DF)_T$ and $(DF)_A$ over a breathing cycle are calculated by superimposing DF for inspiration, deposition efficiency E during pause, and DF for expiration in the tracheobronchial airways and alveolar space. It is assumed that the breathing cycle consists of a constant flow inspiration, a pause, and a constant flow expiration, each with a respective duration fraction of 0.435, 0.05, and 0.515 of a breathing period.

C.5.3. Lung Models

Lung architecture affects particle deposition in several ways: the linear dimension of the airway is related to the distance the particle travels before it contacts the airway surface; the air flow velocity by which the particles are transported is determined by the cross-section of the airway for a given volumetric flowrate; and flow characteristics in the airways are influenced by the airway diameter and branching patterns. Thus, theoretical prediction of particle deposition depends, to a large extent, on the lung model chosen.

C.5.3.1. Lung Model for Rats

Morphometric data on the lung airways of rats were reported by Schum and Yeh (1979). Table C-1 shows the lung model data for Long Evans rats with a total lung capacity of 13.784 cm^3. Application of this model to Fischer rats is accomplished by assuming that the rat has the same lung structure regardless of its strain and that the total lung capacity is proportional to the body weight. In addition, it is also assumed that the lung volume at rest is about 40% of the total lung capacity and that any linear dimension of the lung is proportional to the cubic root of the lung volume.

C.5.3.2. Lung Model for Human Adults

The lung model of mature human adults used in the deposition calculation of DEPs is the symmetric lung model developed by Weibel (1963). In Weibel's model, the airways are assumed to be a dichotomous branching system with 24 generations. Beginning with the 18th generation, increasing numbers of alveoli are present on the wall of the airways and the last three generations are completely aleveolated. Thus, the alveolar region in this model consists of all the airways in the last seven generations. Table C-2 presents the morphometric data of the airways of Weibel's model adjusted to a total lung volume of 3,000 cm^3.

TABLE C-1. LUNG MODEL FOR RATS AT TOTAL LUNG CAPACITY

Generation Number	Number of Airways	Length (cm)	Diameter (cm)	Accumulative Volume[b] (cm)
1	1	2.680	0.340	0.243
2	2	0.715	0.290	0.338
3	3	0.400	0.263	0.403
4	5	0.176	0.203	0.431
5	8	0.208	0.163	0.466
6	14	0.117	0.134	0.486
7	23	0.114	0.123	0.520
8	38	0.130	0.112	0.569
9	65	0.099	0.095	0.615
10	109	0.091	0.087	0.674
11	184	0.096	0.078	0.758
12	309	0.073	0.070	0.845
13	521	0.075	0.058	0.948
14	877	0.060	0.049	1.047
15	1,477	0.055	0.036	1.414
16[a]	2,487	0.035	0.020	1.185
17	4,974	0.029	0.017	1.254
18	9,948	0.025	0.016	1.375
19	19,896	0.022	0.015	1.595
21	39,792	0.020	0.014	2.003
22	79,584	0.019	0.014	2.607
25	318,336	0.017	0.014	7.554
24	636,672	0.017	0.014	13.784

[a]Terminal bronchioles.
[b]Including the attached alveoli volume (number of alveoli = 3×10^7, alveolar diameter = 0.0086 cm).

TABLE C-2. LUNG MODEL BY WEIBEL (1963) ADJUSTED TO 3,000 CM^3 LUNG VOLUME

Generation Number	Number of Airways	Length (cm)	Diameter (cm)	Accumulative Volume[b] (cm)
0	1	10.260	1.539	19.06
2	2	4.070	1.043	25.63
2	4	1.624	0.710	28.63
3	8	0.650	0.479	29.50
4	16	1.086	0.385	31.69
5	32	0.915	0.299	33.75
6	64	0.769	0.239	35.94
7	128	0.650	0.197	38.38
8	256	0.547	0.159	41.13
9	512	0.462	0.132	44.38
10	1,024	0.393	0.111	48.25
11	2,048	0.333	0.093	53.00
12	4,096	0.282	0.081	59.13
13	8,192	0.231	0.070	66.25
14	16,384	0.197	0.063	77.13
15	32,768	0.171	0.056	90.69
16[a]	65,536	0.141	0.051	109.25
17	131,072	0.121	0.046	139.31
18	262,144	0.100	0.043	190.60
19	524,283	0.085	0.040	288.16
20	1,048,579	0.071	0.038	512.94
21	2,097,152	0.060	0.037	925.04
22	4,194,304	0.050	0.035	1,694.16
23	8,388,608	0.043	0.035	3,000.00

[a]Terminal bronchioles.
[b]Including the attached alveoli volume (number of alveoli = 3×10^3, alveolar diameter = 0.0288 cm).

C.5.3.3. Lung Model for Children

The lung model for children in the diesel study was developed by Yu and Xu (1987) on the basis of available morphometric measurements. The model assumes a lung structure with dichotomous branching of airways, and it matches Weibel's model for a subject when evaluated at the age of 25 years, the age at which the lung is considered to be mature. The number and size of airways as functions of age t (years) are determined by the following equations.

C.5.3.3.1. Number of airways and alveoli

The number of airways $N_i(t)$ at generation i for age t is given by

$$N_i(t) = 2^i, \qquad \text{for } 0 \le i \le 20 \tag{C-57}$$

$$\begin{cases} N_{21}(t) = N_r(t), \\ N_{22}(t) = N_{23}(t) = 0. \end{cases} \qquad \text{for } N_r(t) \le 2^{21} \tag{C-58}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = N_r(t) - 2^{21}, \\ N_{23}(t) = 0, \end{cases} \qquad \text{for } 2^{21} < N_r(t) \le 2^{22} \tag{C-59}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = 2^{22}, \\ N_{23}(t) = N_r(t) - 2^{21} - 2^{22} \end{cases} \qquad \text{for } N_r(t) > 2^{21} + 2^{22}, \tag{C-60}$$

where $N_r(t)$ is the total number of airways in the last three airway generations. The empirical equation for N_r which best fits the available data is

$$N_r(t) = \begin{cases} 2.036 \times 10^7 (1 - 0.926 e^{-0.15t}), & t \le 8 \\ 1.468 \times 10^7, & t > 8 \end{cases} \tag{C-61}$$

Thus, $N_r(t)$ increases from approximately 1.5 million at birth to 15 million at 8 years of age and remains nearly constant thereafter. Equations C-58 to C-60 also imply that in the last three generations, the airways in the subsequent generation begin to appear only when those in the preceding generation have completed development.

The number of alveoli as a function of age can be represented by the following equation according to the observed data:

$$N_A(t) = 2.985 \times 10^8 (1 - 0.919 e^{-0.45t}) \tag{C-62}$$

The number of alveoli distributed in the unciliated airways at the airway generation level is determined by assuming that alveolization of airways takes place sequentially in a proximal

direction. For each generation, alveolization is considered to be complete when the number of alveoli in that generation reaches the number determined by Weibel's model.

C.5.3.3.2. Airway size

Four sets of data are used to determine airway size during postnatal growth: (a) total lung volume as a function of age; (b) airway size as given by Weibel's model; (c) the growth pattern of the bronchial airways; and (d) variation in alveolar size with age. From these data, it is found that the lung volume, LV(t) at age t, normalized to Weibel's model at 4,800 cm^3 for an adult (25 years old), follows the equation

$$LV(t) = 0.959 \times 10^5(1 - 0.998e^{-0.002t}) \quad (cm^3). \tag{C-63}$$

The growth patterns of the bronchial airways are determined by the following equations

$$D_i(t) - D_{iw} = \alpha_i[H(t) - H(25)], \tag{C-64}$$

$$L_i(t) - L_{iw} = \beta_i[H(t) - H(25)], \tag{C-65}$$

where $D_i(t)$ and $L_i(t)$ are, respectively, the airway diameter and length at generation i and age t, D_{iw} and L_{iw} the corresponding values for Weibel's model, α_i and β_i are coefficients given by

$$\alpha_i = 3.26 \times 10^{-2}\exp[-1.183\ (i+1)^{0.5}] \tag{C-66}$$

$$\beta_i = 1.05\times10^{-6}\ \exp\ [10.1]\ (i+1)^{-0.2}] \tag{C-67}$$

and H(t) is the body height, which varies with age t in the form

$$H(t) = 1.82 \times 10^2(1 - 0.725e^{-0.14t})\ (cm). \tag{C-68}$$

For the growth patterns of the airways in the alveolar region, it is assumed that

$$\frac{D_i}{D_{iw}} = \frac{L_i}{L_{iw}} = \frac{D_a}{D_{aw}} = f(t), \qquad for\ 17 \le i \le 23 \tag{C-69}$$

where D_a is the diameter of an alveolus at age t, $D_{aw} = 0.0288$ cm is the alveolar diameter for adults in accordance with Weibel's model, and f(t) is a function determined from

$$f(t) = \sqrt[3]{\frac{\{LV(t) - \sum_{i=0}^{16} \frac{\pi}{4} D_i^2(t)\ L_i(t)N_i(t)\}}{\{\sum_{i=17}^{23} \frac{\pi}{4} D_{iw}^2 L_{iw} N_i(t) + \frac{5\pi}{36} D_{aw}^3 N_A(t)\}}} \tag{C-70}$$

C.6. TRANSPORT RATES

The values of transport rates $\lambda_{XY}^{(i)}$ for rats have been derived from the experimental data of clearance for diesel soot (Chan et al., 1981; Strom et al., 1987, 1988) and for the particle associated organics (Sun et al., 1984; Bond et al., 1986; Yu et al., 1991). These values are used in the present model of lung burden calculation and are listed below:

$$\lambda_{HG}^{(i)} = 1.73 \quad (i = 1,2,3) \tag{C-71}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(i)} = 0.00018 \tag{C-72}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \tag{C-73}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \tag{C-74}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{C-75}$$

$$\lambda_{AL}^{(1)} = 0.00068\ [1 - \exp(-0.046 m_A^{1.62})] \tag{C-76}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4}\lambda_{AB}^{(i)} \qquad (i = 2,3) \tag{C-77}$$

$$\lambda_{AT}^{(i)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) \quad (i = 1,2,3) \tag{C-78}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AT}^{(1)} + \lambda_{AB}^{(1)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00086 \tag{C-79}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_a^{1.62}) + 0.0161 \tag{C-80}$$

$$\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) + 15.7 \tag{C-81}$$

where $\lambda_{XY}^{(i)}$ is the unit of day^{-1}, and $m_A \cong m_A^{(1)}$ is the particle burden (in mg) in the alveolar compartment.

Experimental data on the deposition and clearance of DEPs in humans are not available. To estimate the lung burden of DEPs for human exposure, it is necessary to extrapolate the transport rates $\lambda_{XY}^{(i)}$ from rats to humans. For organics, we assume that the transport rates are the same for rats and humans. This assumption is based upon the observation of Schanker et al. (1986) that the lung clearance of inhaled lipophilic compounds appears to depend only on their

lipid/water partition coefficients and is independent of species. In contrast, the transport rates of diesel soot in humans should be different from that of rats, since the alveolar clearance rate, λ_A, of insoluble particles at low lung burdens for human adults is approximately seven times that of rats (Bailey et al., 1982), as previously discussed in Chapter 4.

No data are available on the change of the alveolar clearance rate of insoluble particles in humans due to excessive lung burdens. It is seen from equation C-79 that $\lambda_A^{(1)}$ for rats can be written in the form

$$\lambda_A^{(1)} = a \exp(-b m_A^c) + d \qquad \text{(C-82)}$$

where a, b, c, and d are constants. The right-hand side of equation C-82 consists of two terms, representing, respectively, macrophage-mediated mechanical clearance and clearance by dissolution. The first term depends upon the lung burden, whereas the second term does not. To extrapolate this relationship to humans, we assume that the dissolution clearance term was independent of species and that the mechanical clearance term for humans varies in the same proportion as in rats under the same unit surface particulate dose. This assumption results in the following expression for $\lambda_A^{(1)}$ in humans

$$\lambda_A^{(1)} = \frac{a}{P} \exp(-b(m_A/S)^c) + d \qquad \text{(C-83)}$$

where P is a constant derived from the human/rat ratio of the alveolar clearance rate at low lung burdens, and S is the ratio of the pulmonary surface area between humans and rats. equation C-83 implies that rats and humans have equivalent amounts of biological response in the lung to the same specific surface dose of inhaled DEPs.

From the data of Bailey et al. (1982), we obtain a value of $\lambda_A^{(1)} = 0.00169$ day^{-1} for humans at low lung burdens. This leads to P = 14.4. Also, we find S=148 from the data of the anatomical lung model of Schum and Yeh (1979) for rats and Weibel's model for human adults. For humans less than 25 years old, we assume the same value for P, but S is computed from the data of the lung model for young humans (Yu and Xu 1987). The value of S for different ages is shown in Table C-3.

The equations for other transport rates that have a lung-burden-dependent component are extrapolated from rats to humans in a similar manner. The following lists the values of $\lambda_{XY}^{(i)}$ (in day^{-1}) for humans used in the present model calculation:

$$\lambda_{HG}^{(1)} = 1.73 \quad (i = 1,2,3) \qquad \text{(C-84)}$$

TABLE C-3. RATIO OF PULMONARY SURFACE AREAS BETWEEN HUMANS AND RATS AS A FUNCTION OF HUMAN AGE

Age (Year)	Surface Area
0	4.99
1	17.3
2	27.6
3	36.7
4	44.7
5	51.9
6	58.5
7	64.6
8	70.4
9	76.0
10	81.4
11	86.6
12	91.6
13	96.4
14	101
15	106
16	110
27	115
28	119
19	123
20	128
21	132
22	136
23	140
24	144
25	148

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(1)} = 0.00018 \quad \text{(C-85)}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \quad \text{(C-86)}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \quad \text{(C-87)}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \quad \text{(C-88)}$$

$$\lambda_{AL}^{(1)} = 0.00068 \{1 - 0.0694 \exp[-0.046(m_A/S)^{1.62}]\} \quad \text{(C-89)}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4} \lambda_{AB}^{(i)} \qquad (i = 2, 3) \quad \text{(C-90)}$$

$$\lambda_{AT}^{(i)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} \quad (i = 1, 2, 3) \quad \text{(C-91)}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AB}^{(1)} + \lambda_{AT}^{(1)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}]\} + 0.00086 \quad \text{(C-92)}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.0694\{0.012 \exp[-0.11(m_A/A)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} + 0.016 \quad \text{(C-93)}$$

$$\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}\} + 15.7 \quad \text{(C-94)}$$

C.7. RESULTS

C.7.1. Simulation of Rat Experiments

To test the accuracy of the model, simulation results are obtained on the retention of diesel soot in the rat lung and compare with the data of lung burden and lymph node burden obtained by Strom et al. (1988). A particle size of 0.19 μm MMAD and a standard geometric deviation, σ_g of 2.3 (as used in Strom's experiment) are used in the calculation.

The respiratory parameters for rats are based on their weight and calculated using the following correlations of minute volume, respiratory frequency, and growth curve data.

$$\text{Minute Volume} = 0.9W \ (\text{cm}^3/\text{min}) \quad \text{(C-95)}$$

$$\text{Respiratory Frequency} = 475W^{-0.3} \ (1/\text{min}) \quad \text{(C-96)}$$

where W is the body weight (in grams) as determined from the equation

$$W = 5+537T/(100+T), \text{ for } T \geq 56 \text{ days} \quad \text{(C-97)}$$

in which T is the age of the rat measured in days.

Equation C-95 was obtained from the data of Mauderly (1986) for rats ranging in age from 3 mo to 2 years old; equation C-96 was obtained from the data of Strom et al. (1988); and equation C-59 was determined from the best fit of the experimental deposition data. Figures C-3 and C-4 show the calculated lung burden of diesel soot ($m_A^{(1)} + m_D^{(1)}$ and lymph node burden, respectively, for the experiment by Strom et al. (1988) using animals exposed to DEPs at 6 mg/m^3 for 1,3,6 and 12 weeks; exposed in all cases was 7 days/week and 20 h daily. The solid lines represent the calculated accumulation of particles during the continuous exposure phase and the dashed lines indicate calculated post exposure retention. The agreement between the calculated and the experimental data for both lung and lymph node burdens during and after the exposure periods was very good.

Comparison of the model calculation and the retention data of particle-associated BaP in rats obtained by Sun et al. (1984) is shown in Figure C-5. The calculated retention is shown by the solid line. The experiment of Sun et al. consisted of a 30 min exposure to diesel particles coated with [^3H] benzo[a]pyrene (*[^3H] - BaP*) at a concentration of 4 to 6 μg/m^3 of air and followed by a post exposure period of over 25 days. The fast and slow phase of (*[^3H] - BaP*) clearance half-times were found to be 0.03 day and 18 days, respectively. These correspond to $\lambda_{AO}^{2} = 0.0385$ day^{-1} and $\lambda_{AO}^{(3)} = 23.1$ day^{-1} in our model, where $\lambda_{AO}^{(i)}$ is the value of $\lambda_{XY}^{(i)}$ at $m_A \rightarrow 0$. Figure C-5 shows that the calculated retention is in excellent agreement with the experimental data obtained by Sun et al. (1984).

C.7.2. Predicted Burdens in Humans

Selected results of lung burden predictions in humans are shown in Figures C-6 to C-9. The particle conditions used in the calculation are 0.2 μm MMAD with $\sigma_g = 2.3$, and the mass fractions of the rapidly and slowly cleared organics are each 10% ($f_1 = f_2 = 0.1$). Figures C-6 and C-7 show, respectively, the lung burdens per unit concentration of diesel soot and the associated organics in human adults for different exposure patterns at two soot concentrations, 0.1 and 1 mg/m^3. The exposure patterns used in the calculation are (a) 24 h/day and 7 days week; (b) 12 h/day and 7 days/week; and (c) 8 h/day and 5 days/week, simulating environmental and occupational exposure conditions. The results show that the lung burdens of both diesel soot and the associated organics reached a steady state value during exposure. Due to differences in the amount of particle intake, the steady state lung burdens per unit concentration were the highest for exposure pattern (a) and the lowest for exposure pattern (b). Also, increasing soot concentratiion from 0.1 to 1 mg/m^3 increased the lung burden per unit concentration. However, the increase was not noticeable for exposure pattern (c). The dependence of lung burden on the soot concentration is caused by the reduction of the alveolar clearance rate at high lung burdens discussed above.

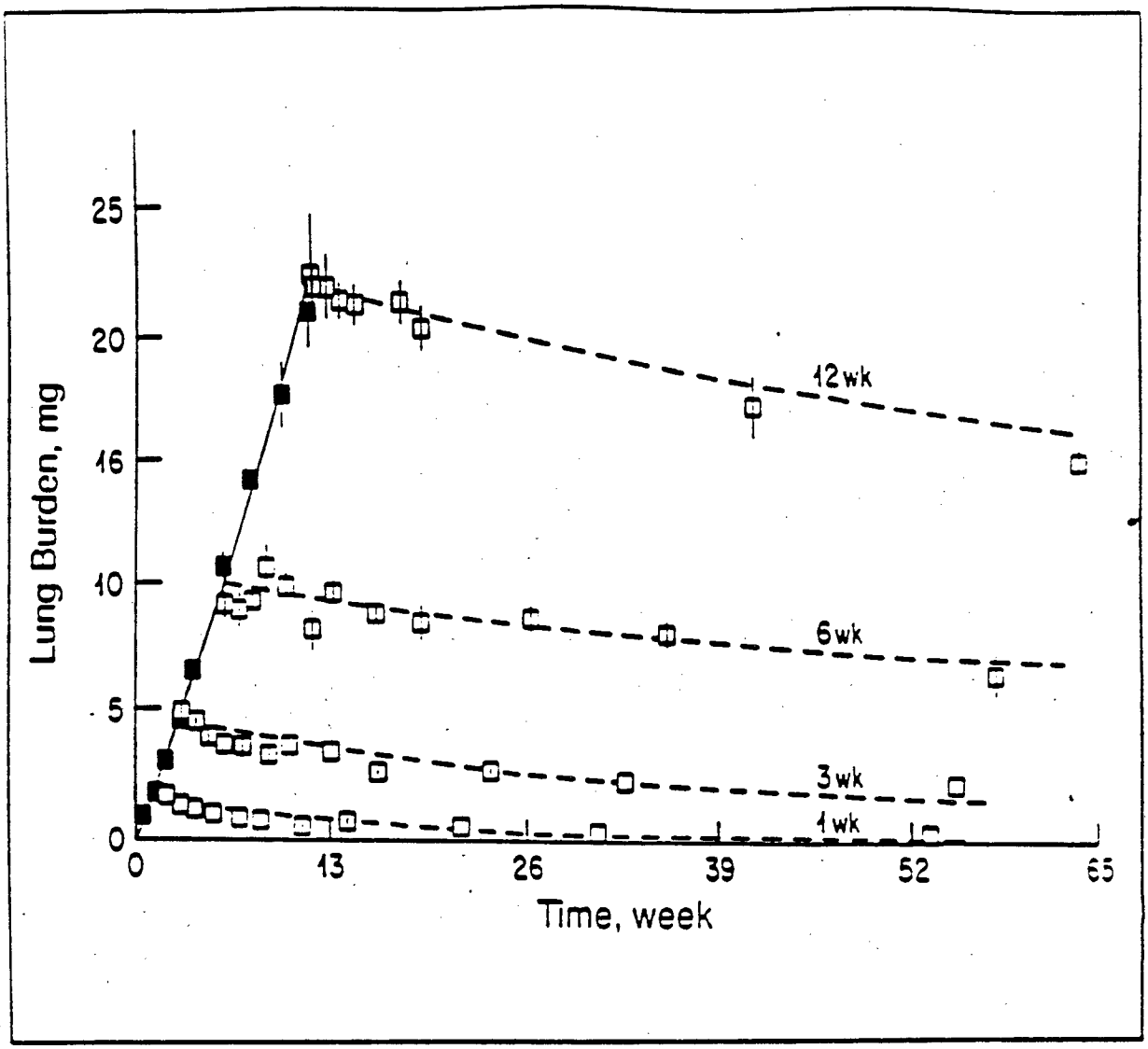

Figure C-3. The experimental and predicted lung burdens of rats to DEPs at a solid and dashed concentration of 0.6 mg/m^3 for different exposure spans. Lines are, respectively, the predicted burdens during exposure and post exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

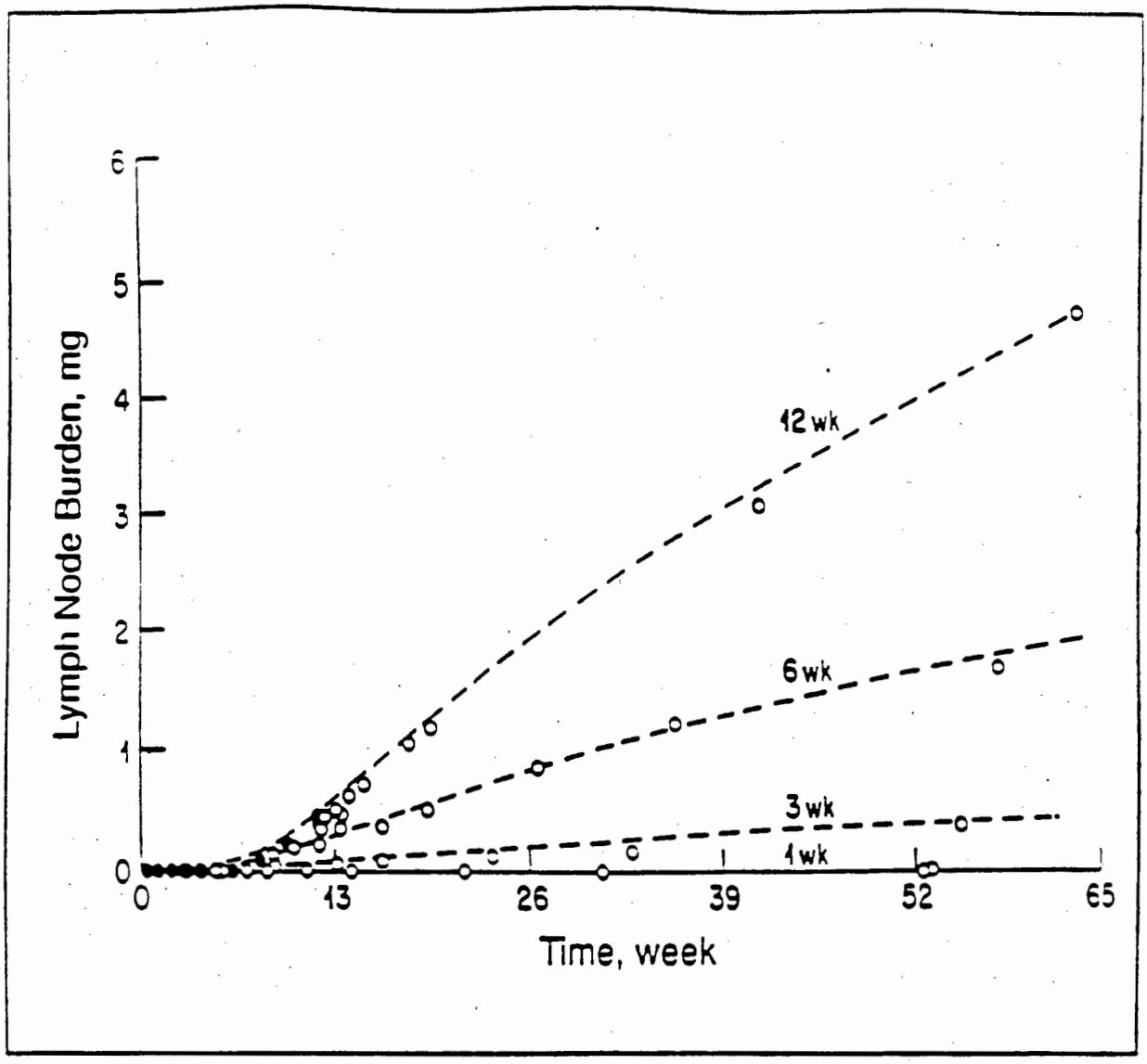

Figure C-4. Experimental and predicted lymph node burdens of rats exposed to CEPs at a concentration of 6.0 mg/m^3 for different exposure spans. The solid and dashed lines are, respectively, the predicted burdens during exposure and post exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

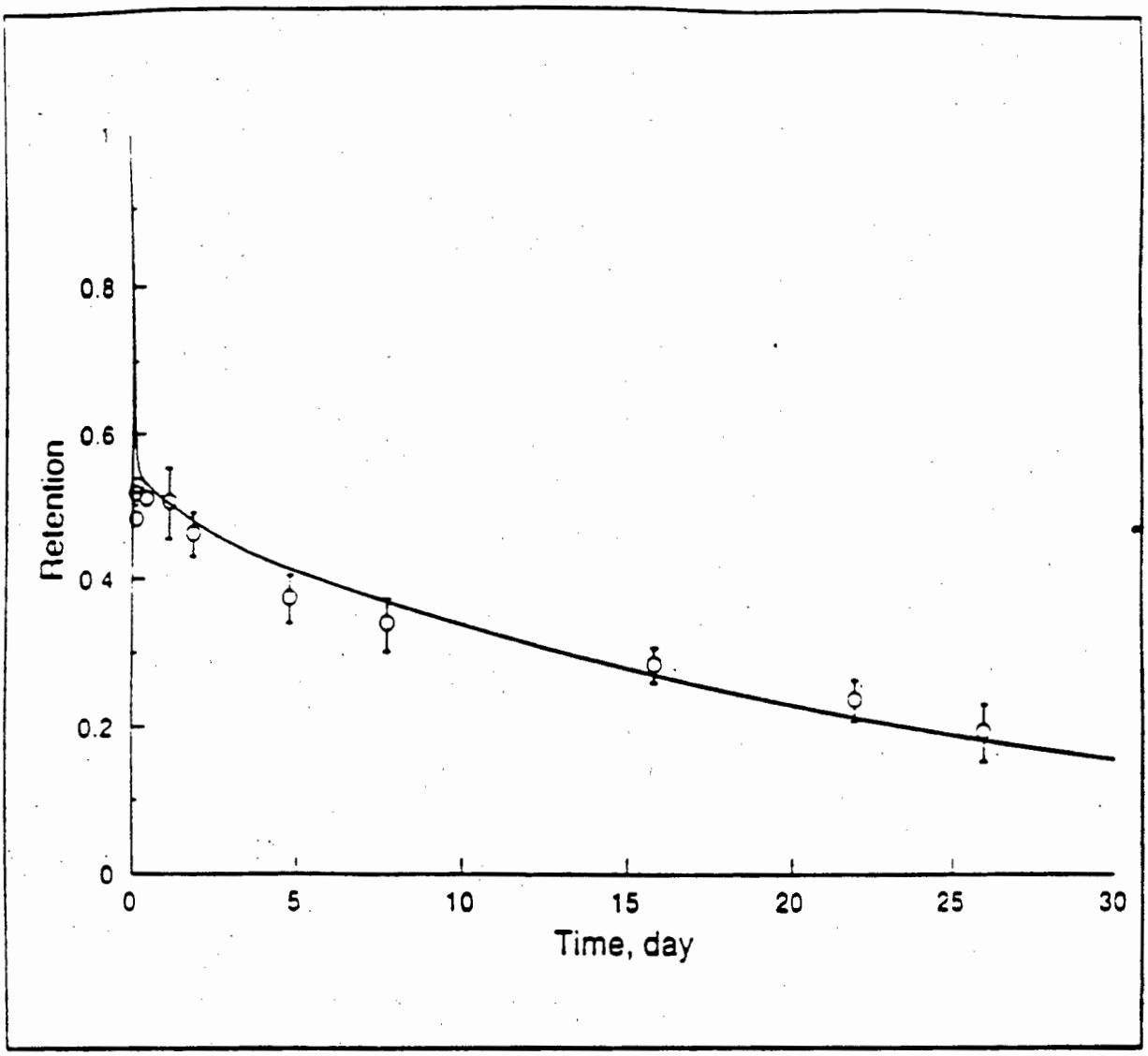

Figure C-5. Comparison between the calculated lung retention (solid line) and the experimental data obtained by Sun et al. (1984) for the particle associated BaP in rats.

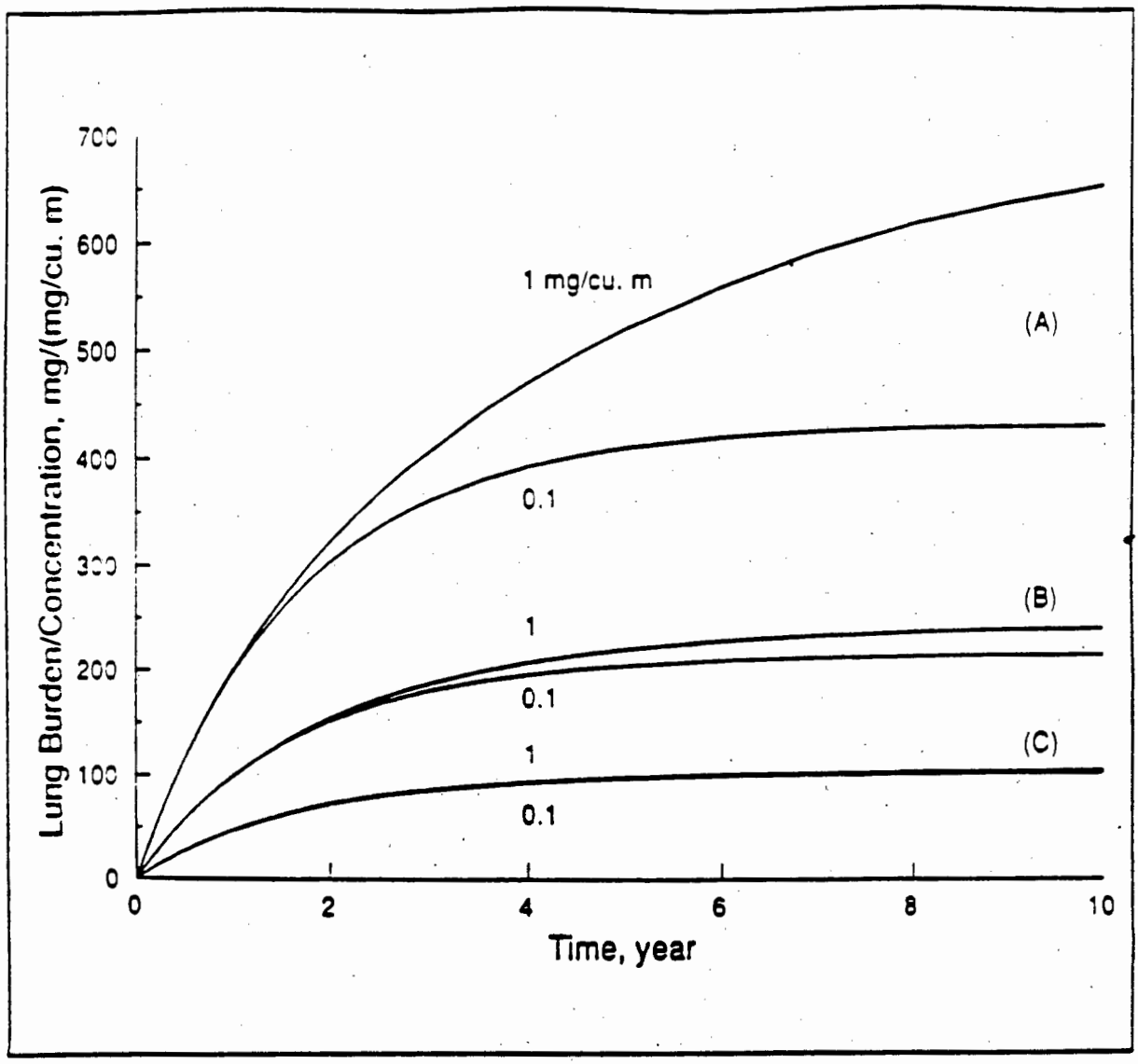

Figure C-6. Calculated lung burdens of diesel soot per unit exposure concentration in human adults exposed continuously to DEPs at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

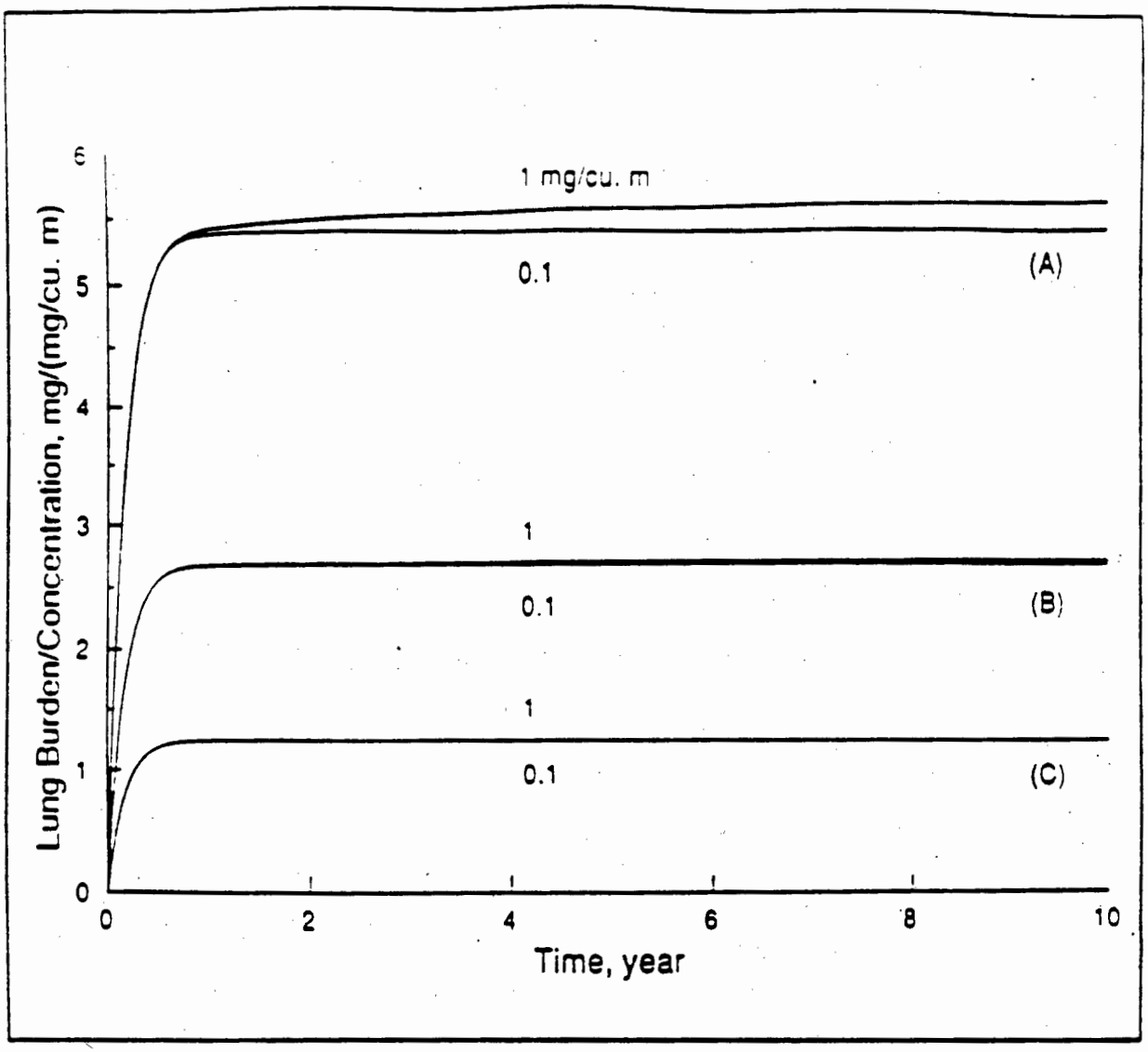

Figure C-7. Calculated lung burdens of the particle-associated organics per unit exposure concentration in human adults exposed continuously to DEPs at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

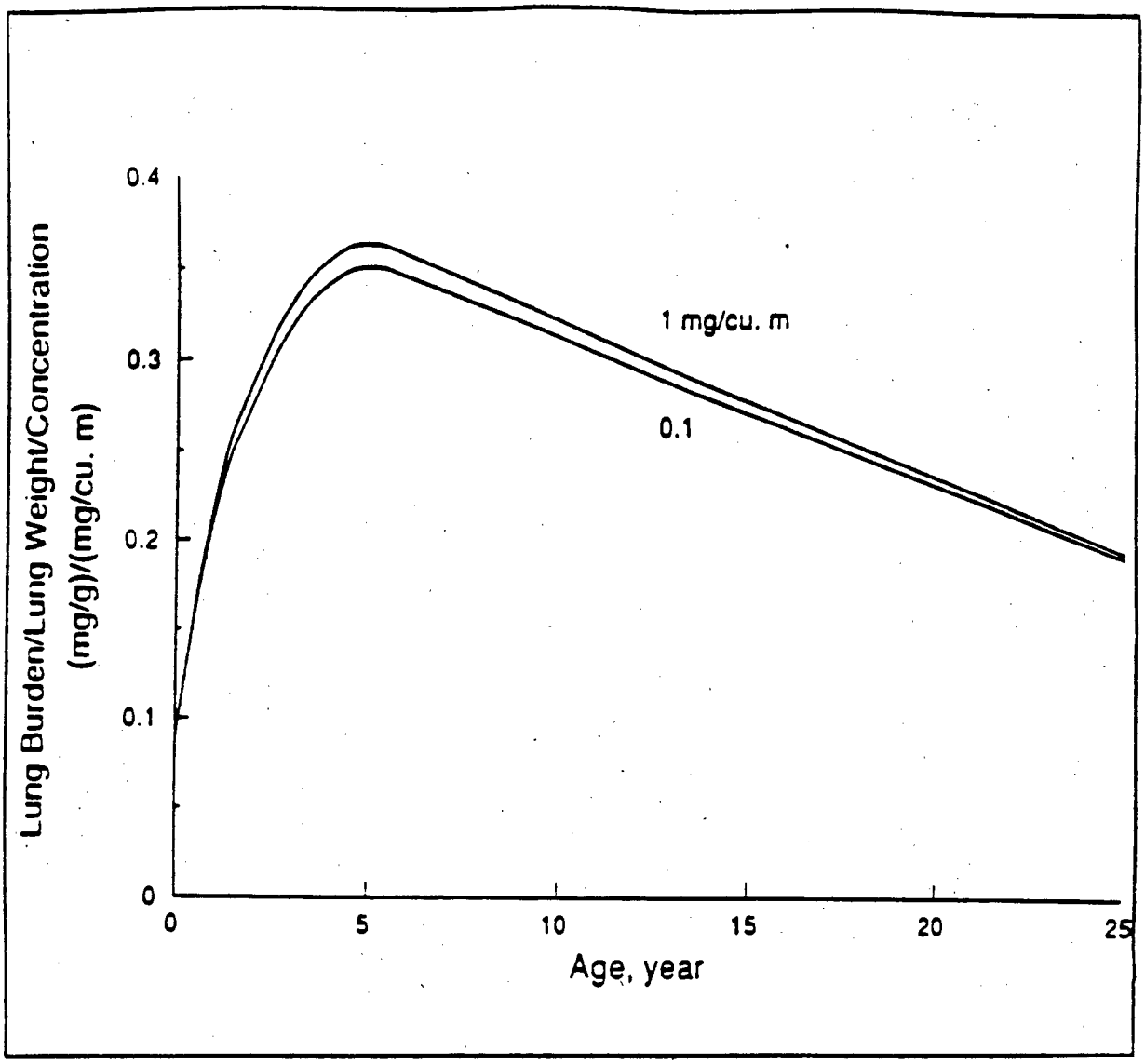

Figure C-8. Calculated lung burdens of diesel soot per gram of lung per unit exposure concentration in humans of different ages exposed continuously for one year to DEPs of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.

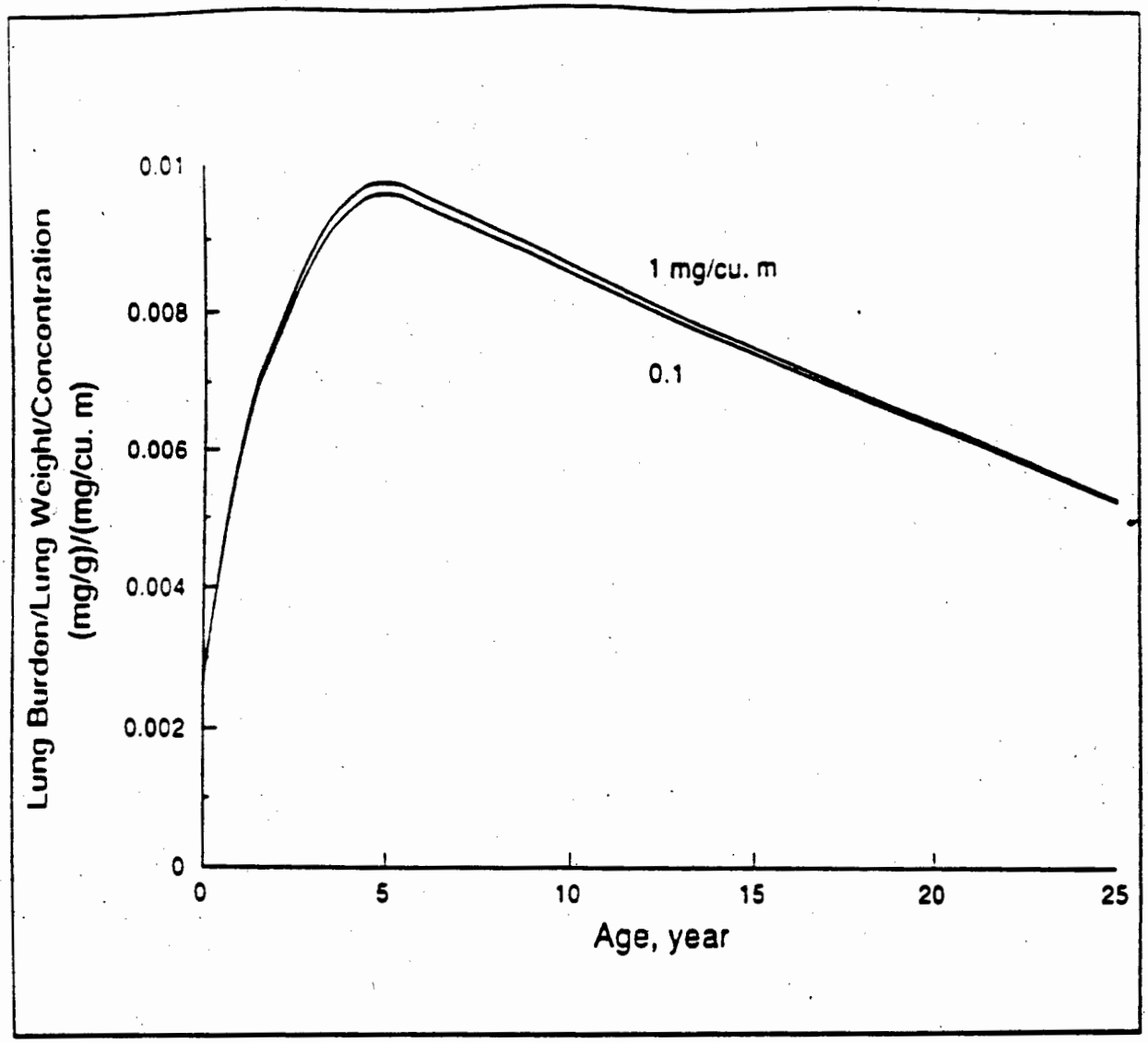

Figure C-9. Calculated burdens of the particle-associated organics per gram of lung per unit exposure concentration in humans of different ages exposed continuously for one year to DEPs of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.

Figures C-8 and C-9 show the effect of age on lung burden, where the lung burdens per unit concentration per unit weight are plotted versus age. The data of lung weight at different ages are those reported by Snyder (1975). The exposure pattern used in the calculation is 24 h/day and 7 days/week for a period of one year at the two soot concentrations, 0.1 and 1 mg/m^3. The results show that, on a unit lung weight basis, the lung burdens of both soot and the organics are functions of age and the maximum lung burdens occur at approximately 5 years of age. Again, for any given age, the lung burden per unit concentration is slightly higher at 1 mg/m^3 than at 0.1 mg/m^3.

C.8. PARAMETRIC STUDY OF THE MODEL

The deposition and clearance model of DEPs in humans, presented above, consists of a large number of parameters which characterize: the size and composition of diesel particles, the structure and dimension of the respiratory tract, the ventilation conditions of the subject, and the clearance half-times of the diesel soot and the particle-associated organics. Any single or combined changes of these parameters from their normal values in the model would result in a change in the predicted lung burden. A parametric study has been conducted to investigate the effects of each individual parameter on calculated lung burden in human adults. The exposure pattern chosen for this study is 24 h/day and 7 days/week for a period of 10 years at a constant soot concentration of 0.1 mg/m^3. The following presents two important results from the parametric study.

C.8.1. Effect of Ventilation Conditions

The change in lung burden due to variations in tidal volume and respiratory frequency are depicted in Figures C-10 and C-11. Increasing any one of these ventilation parameters increased the lung burden, but the increase was much smaller with respect to respiratory frequency than to tidal volume. This small increase in lung burden was a result of the decrease in deposition efficiency as respiratory frequency increased, despite a higher total amount of DEPs inhaled.

The mode of breathing has only a minor effect on lung burden because switching from nose breathing does not produce any appreciable change in the amount of particle intake into the lung (Yu and Xu, 1987). All lung burden results presented in this report are for nose breathing.

C.8.2. Effect of Transport Rates

Transport rates have an obvious effect on the retention of DEPs in the lung after deposition. Because we are mainly concerned with the long-term clearance of diesel soot and the associated organics, only the effects of two transport rates $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ are studied.

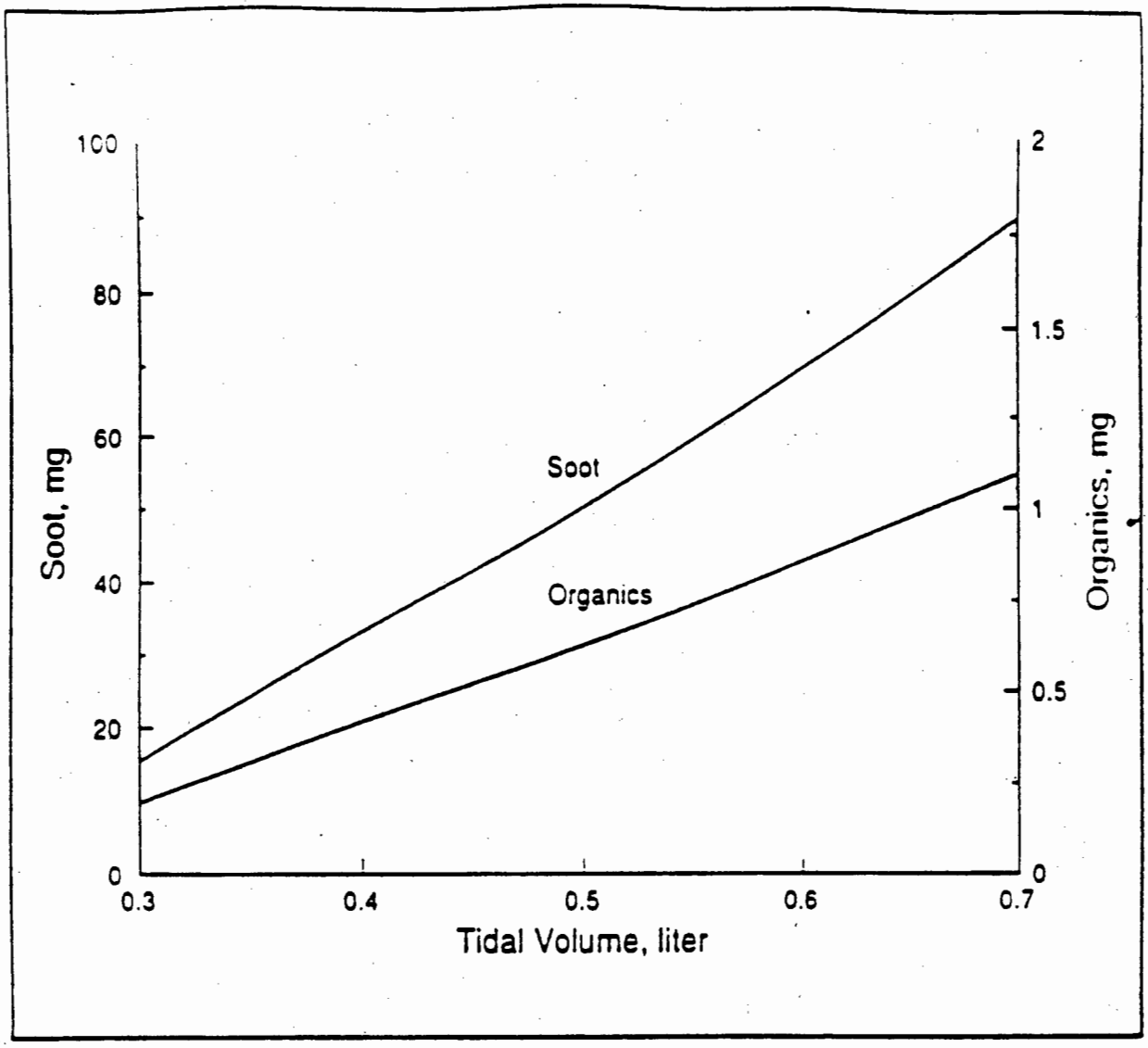

Figure C-10. Calculated lung burdens in human adults versus tidal volume in liter for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1, (b) respiratory frequency = 14 min^{-1}, and (c) lung volume = 3000 cm^3.

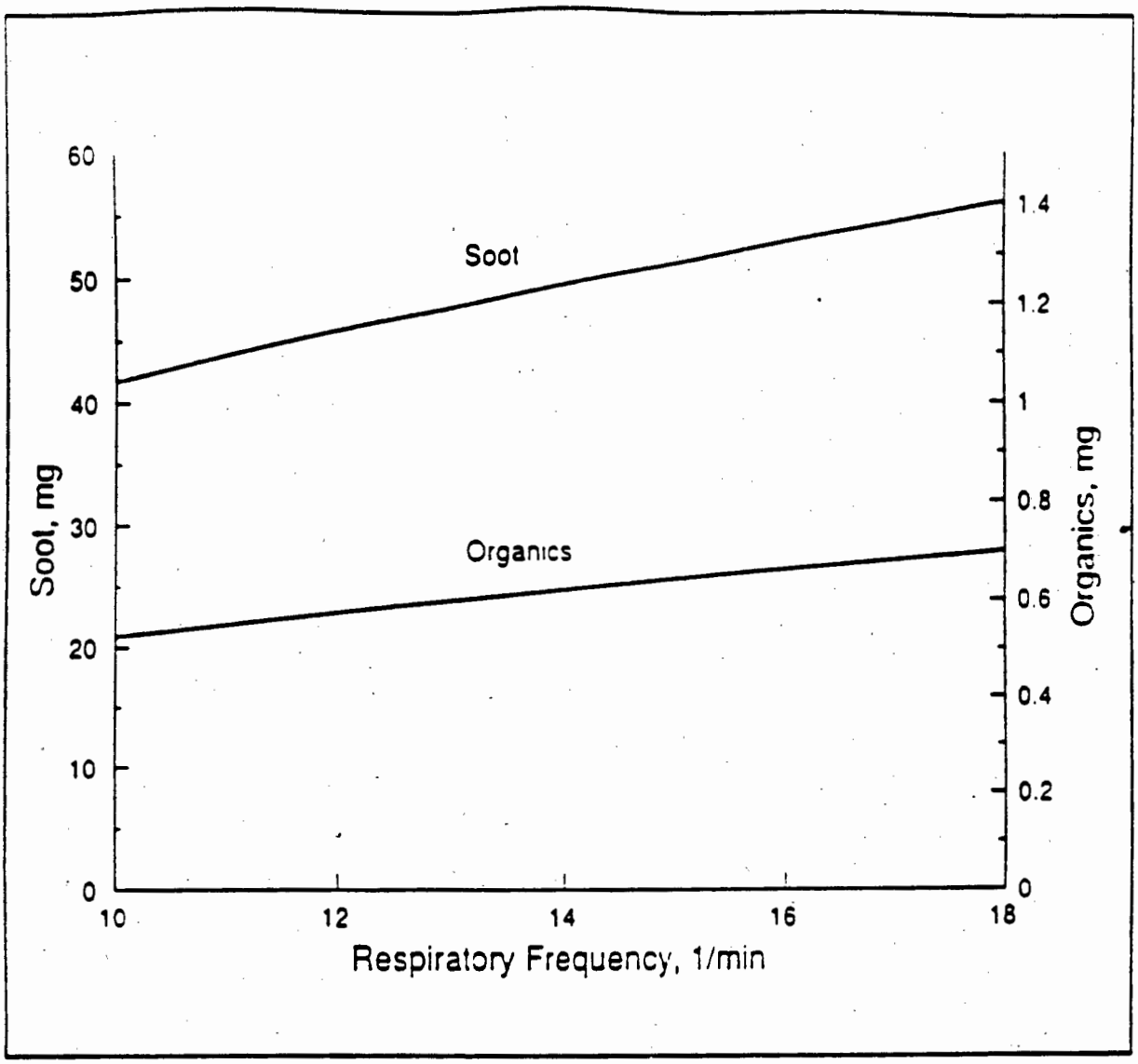

Figure C-11. **Calculated lung burdens in human adults versus respiratory frequency in *bpm* for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1, (b) tidal volume = 500 cm^3, and (c) lung volume = 3200 cm^3.**

Experimental data of $\lambda_A^{(1)}$ from various diesel studies in rats have shown that $\lambda_A^{(1)}$ can vary by a factor of two or higher. We use a multiple of 0.5 to 2 for the uncertainty in $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ to examine the effect on lung burden. Figures C-12 and C-13 show respectively, the lung burden results for diesel soot and the associated organics versus the multiples of $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ used in the calculation. As expected, increasing the multiple of $\lambda_A^{(1)}$ reduced the lung burden of diesel soot with practically no change in the organics burden (Figure C-12), while just the opposite occurred when the multiple of $\lambda_A^{(2)}$ was increased (Figure C-13).

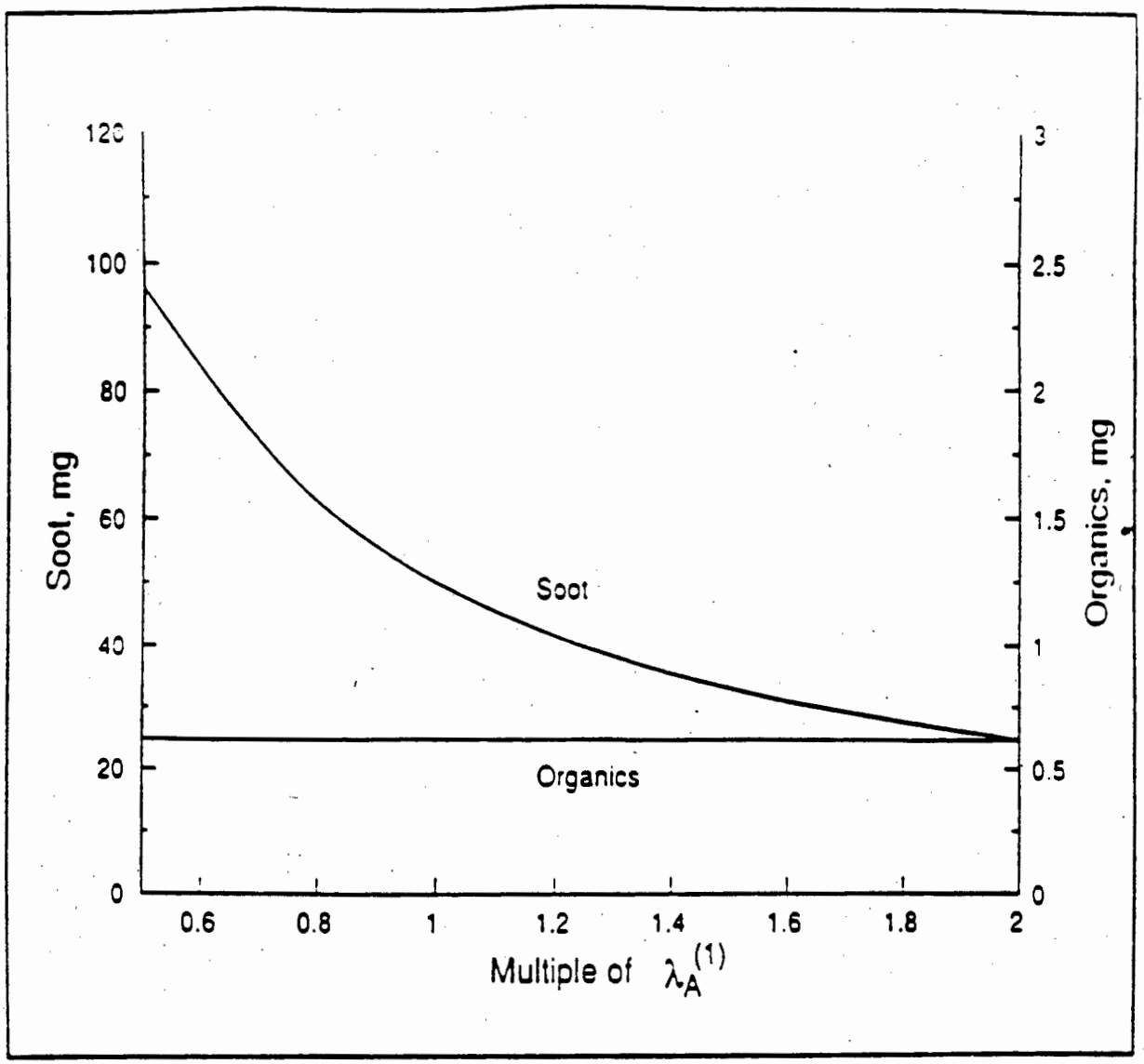

Figure C-12. **Calculated lung burdens in human adults versus multiple of $\lambda_A^{(I)}$ for exposure to DEPs at 0.1 mg/m$_3$ for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1, (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}, and (c) lung volume = 3,200 cm^3.**

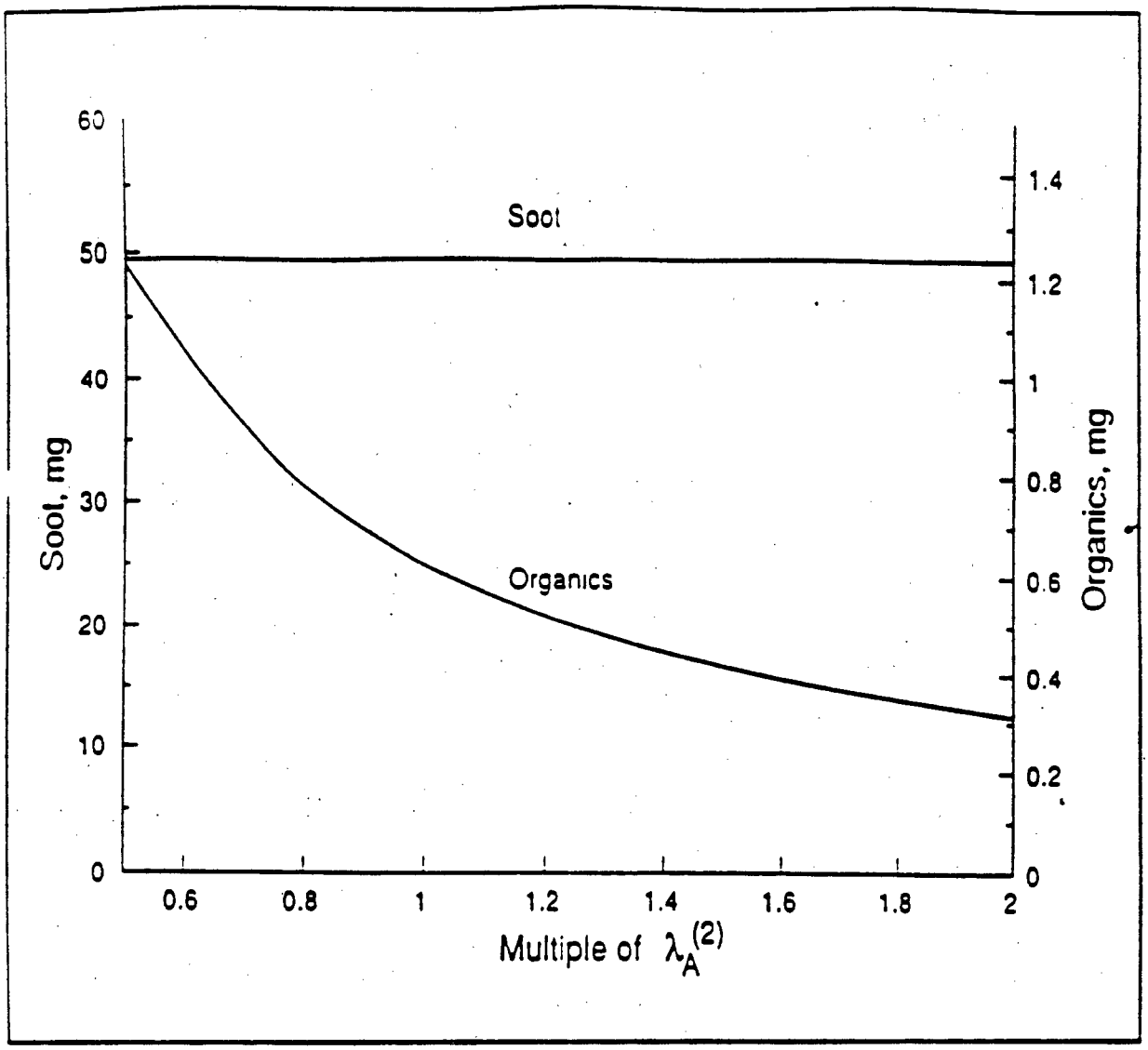

Figure C-13. **Calculated lung burdens in human adults versus multiple of $\lambda_A^{(1)}$ for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm σ_g=2.3, f_2=0.1, f_3=0.1, (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}, and (c) lung volume = 3,200 cm^3.**

C.9. REFERENCES

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	
EPA/600/8-90/057C		

4. TITLE AND SUBTITLE	5. REPORT DATE
Health Assessment Document for Diesel Emissions (SAB Review Draft)	February 1998
	6. PERFORMING ORGANIZATION CODE: EPA/600/021
7. AUTHOR(S): See Author's Page	8. PERFORMING ORGANIZATION REPORT NO.: NCEA-W-0114
9. PERFORMING ORGANIZATION NAME AND ADDRESS: National Center for Environmental Assessment-Washington Office of Research and Development U.S. Environmental Protection Agency Washington, DC 20460	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED: Health Assessment
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The U.S. EPA has prepared an assessment of the possible health hazards from human exposure to diesel engine exhaust emissions. This 1998 assessment is an update of a December 1994 assessment which was reviewed in 1995 by the Agency's Clean Air Scientific Advisory Committee (CASAC). The 1998 assessment focuses on health hazards (hazard identification and dose-response analysis for the purpose of recommending measures of risk/hazard), and also provides background information about diesel engine emissions that is useful for putting the health information into context. EPA risk assessment methods and practice have been followed in identifying possible human chronic health hazards for adverse noncancer effects as well as carcinogenicity hazards. This assessment will under go a second review by the CASAC in the Spring 1998, and following a consideration of comments, a final document will be issued later in 1998. Notice of the CASAC review, opportunity for public comment, and availability of the draft document will be made in a separate notice. The assessment document will be available via internet (www.epa.gov/ncea) and from NTIS in mid to late March, 1998.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to the Public	Unclassified	573
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

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