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Health Assessment Document for Diesel Emissions

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PREFACE

This draft health risk assessment document was prepared by the National Center for Environmental Assessment (NCEA), which is the health risk assessment program in EPA's Office of Research and Development. The assessment has been prepared for EPA's Office of Mobile Sources which requested advice regarding the potential health hazards associated with diesel engine use. As diesel exhaust emissions also affect air toxics and ambient particulate matter, other EPA air programs also have an interest in this assessment. The previous draft of this assessment was released for public comment in February 1998, and the Agency's Clean Air Scientific Advisory Committee (CASAC) met in public session in May 1998 to review the draft. This November 1999 draft is a revision of that 1998 draft, but also builds on the 1990-1999 history of the development of this diesel health risk assessment.

The scientific literature search for this assessment is generally current through January 1999, though a few more recent publications on key topics also have been included.

This November 1999 draft assessment will be reviewed by CASAC in December 1999, and concurrently, public comments will be accepted for a limited time. Following the receipt of comments from CASAC and the public, NCEA plans to finalize the assessment.

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This document was preceded by three earlier drafts: a Workshop Review Draft (EPA/600/8-90/057A, July 1990), an External Review Draft (EPA/600/8-90/057B, December 1994), and an SAB Review Draft (EPA/600/8-90/057C, February 1998). The Science Advisory Board's Clean Air Scientific Advisory Committee (CASAC) reviewed the 1994 draft in public sessions in May 1995 and the 1998 draft in May 1998. Public comment periods also were conducted concurrently with the CASAC reviews. In addition, many reviewers both within and outside the Agency provided assistance at various review stages.

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1. EXECUTIVE SUMMARY

The Health Assessment Document for Diesel Emissions represents the Agency's first comprehensive review of health effects from exposure to exhaust from diesel engines. In-depth research on diesel exhaust (DE) started in the 1970s, and EPA began regulating emission levels for certain types of diesel engines during the same period. EPA wanted to be aware of the current health issues as it continues with Clean Air Act regulatory programs, hence the need for this assessment. In nine chapters, this health assessment addresses key themes or questions such as (1) the health effects of concern for humans, (2) the best insight as to the mode of action and measure of dose/exposure for the toxic response(s), (3) what dose-response analysis suggests about the possible impact/risk to a human population, and (4) the overall nature of the hazard and the related confidence or uncertainties.

Diesel exhaust is a complex mixture of particles and gases with hundreds of chemical compounds, including many organic compounds, present on the particles and in the gases. The particles have an elemental carbon core, with individual particles being very small (a mean aerodynamic diameter of about 0.2 μm) and thus highly respirable. The small particles have a large surface area upon which many organic compounds are adsorbed. The particle organics generally contribute 10%-30% of particle weight and, for example, contain various types of polyaromatic hydrocarbons (PAHs). The gases have both inorganic and organic constituents (e.g., sulfur dioxide, nitrogen oxides, benzene, ethylene, toluene, aldehydes, olefins, and low-molecular-mass PAHs). Both the particles and the numerous organic compounds of DE have toxicological properties that are capable of influencing a toxic response in humans, though the role of either or both in producing a toxic effect in humans is unknown.

DE particles contribute to ambient particulate matter, e.g., $PM_{2.5}$. Compared to other sources of ambient PM, the elemental carbon core is nearly unique to DE, as are a few of the adsorbed organic compounds. The DE gases are more ubiquitous in an urban environment.

Diesel engines may be on-road (vehicle engines) or off-road (many types of engines powering equipment, machinery, railroad locomotives, and ships). Quantitatively, amounts of specific emission constituents vary by type of engine and even within the same engine type. Qualitatively, the basic composition is fairly consistent, for example, an elemental carbon core particle with PAHs adsorbed to the particle and also present in the gases. Over the years, the mass of particles emitted in engine exhaust has been reduced, as have the accompanying organics.

For years researchers have measured DE concentrations using particle mass per unit volume, i.e., $\mu g/m^3$ of diesel particulate matter. This assessment adopts $\mu g/m^3$ as a dosimeter and further assumes that the important toxicologic agents in DE will be proportional to $\mu g/m^3$. This leads to some uncertainty, but the best dosimeter will not be known until the mode of action for DE toxicity is better understood. Questions have been raised as to whether toxicological findings generated from exposure to older engine exhaust can appropriately be applied to current-day engine exhaust exposures. This question is not resolvable with present information, except to note that available evidence does not point to significant shifts in DE composition relative to the total organics over the years, and that organics are believed to be in relative proportion to the mass of particles.

The primary chronic health concerns include nonmalignant respiratory effects and lung carcinogenicity. The DE particulates can be a component of ambient $PM_{2.5}$. Compared to ambient $PM_{2.5}$ with no DE component, DE is likely to have a higher proportion of fine and ultrafine particulates and is likely to have a higher or at least a varied content of toxicologically active organic compounds. Although some similarities exist between DE and ambient PM, the differences are potentially significant. A comparison of the DE RfC and the $PM_{2.5}$ standard has considerable complexity. For ambient PM we see increased mortality and morbidity in human studies from various forms of chronic respiratory disease. For DE we expect adverse respiratory effects but have not clearly observed them in human studies, possibly because few such studies have focused on respiratory effects. Animal studies conducted at higher than ambient exposure levels, the most prominent being in the rat, provide the basis for the expectation of human respiratory disease. A recommended human chronic exposure level without appreciable hazard (i.e., inhalation Reference Concentration, RfC, 5 $\mu g/m^3$) from adverse noncancer respiratory effects is provided in the assessment. From an acute exposure standpoint, DE is an irritant to the respiratory system given sufficient episodic exposure and may cause a variety of inflammation-related symptoms (e.g., headache, eye discomfort, asthma-like reactions, nausea, etc.) depending on individual susceptibility to the DE constituents. Data also suggest that DE is a factor in exacerbating or initiating allergenic hypersensitivity; this is an emerging area of concern.

The carcinogenicity of DE also has been of research and public health interest. Diesel engine exhaust is "highly likely" to be carcinogenic by the inhalation route of exposure, according to EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment. This hazard is viewed as being applicable to ambient (i.e., environmental) exposures. Many of the organics present on the DE particles and in the gases, though in small quantities, are mutagenic and/or

carcinogenic in their own right. DE shows a pattern of statistically increased lung cancer in more than 20, but not all, human occupational studies where DE exposure is prominent. Lung cancer increases are, on average, about 33-47% above background levels, though specific studies suggest some modestly higher increases. There are some uncertainties about the magnitude of the increase, because questions about exposure are almost always present in the human studies in which the increases are seen, and with lung cancer, the question of confounding by cigarette smoke is present. Nevertheless, analysis of the occupational studies shows that the pattern of increased lung cancer remains after consideration of these issues. Bladder cancer also has been elevated in some epidemiologic studies, though the totality of the evidence is too weak to form a clear conclusion. Although rat inhalation cancer bioassays were once thought to be useful for inferring a human cancer hazard or supporting human evidence, in recent years, the rat lung cancer responses seen with DE exposure are thought to be less clear for human hazard prediction and unsuitable for environmental exposure risk estimation. None of the available studies show that the lung cancer hazard is present at environmental levels of exposure, although the margin may be relatively small between some higher environmental exposures and occupational exposures where lung cancer risks are thought to be present.

The plausibility of an environmental lung cancer hazard from DE by inhalation exposure is supported by findings contained in this assessment. Overall, the evidence for a likely human lung cancer hazard by inhalation is persuasive, even though, in the absence of complete data, inferences and thus uncertainties are involved. Some of the key uncertainties include: (1) methodologic limitations inherent in epidemiologic studies, as well as a lack of reliable historical exposure data for occupationally exposed cohorts, (2) uncertainties regarding the extent of bioavailability of organic compounds present on diesel particles and their impact on the carcinogenic process, and (3) other uncertainties regarding the mode of action of DE on lung cancer in humans.

A decision has been made in this assessment that, despite the finding that DE is best characterized as highly likely to be a lung cancer hazard, the available data are currently unsuitable to make a confident quantitative statement about the magnitude of the lung cancer risk attributable to DE at ambient exposure levels. Therefore, this assessment does not adopt or recommend a specific cancer unit risk estimate for DE. However, information is provided to put DE cancer hazard in perspective and to assist decisionmakers and the public to make prudent public health judgments in the absence of a definitive estimate of the upper bound on cancer risk. Efforts to derive cancer risk estimates for environmental purposes continue, with the focus being

on epidemiologic studies because the epidemiology-based estimates are always the ideal starting point, while also recognizing that the rat inhalation studies are no longer favored and other approaches identified to date have limitations.

There is no DE-specific information that provides direct insight to the question of variable susceptibility within the population. Default approaches to account for uncertainty in inter-individual susceptibility have been included in the derivation of the RfC. Individuals with preexisting lung burdens of particulates may have less of a margin of safety from DE particulate-driven hazards than might be inferred from incremental DE exposure analysis, although this cannot be quantified. DE exposure could be additive to many other daily or lifetime exposures to organics and PM. 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 could be more susceptible to adverse impacts from DE exposure. Although there is no information from studies of DE, infants and children could have a greater susceptibility to the acute/chronic toxicity of DE because they have greater ventilatory frequency, resulting in greater respiratory tract particle deposition. The issue of DE impacts on allergenicity and potential onset and exacerbation of childhood asthma is being actively investigated, but firm conclusions await peer review and publication of ongoing work.

Another aspect of differential susceptibility involves subgroups that may receive additional exposure to DE because of their proximity to DE sources. Those having outside time in their daily routine and being near a diesel emission source would likely receive more exposure than others in the population. The highest exposed are most likely the occupational subgroups whose job brings them very close to diesel emission sources (e.g., trucking industry, machinery operations, engine mechanics, some types of transit operations, railroads, etc.).

Ongoing analyses by EPA, other Federal agencies, and worldwide researchers are expected to improve the existing epidemiology and related exposure databases. These will provide new opportunities to evaluate the potential health effects of DE on the general population and susceptible subgroups.

2. DIESEL EMISSIONS CHARACTERIZATION, ATMOSPHERIC TRANSFORMATION, AND EXPOSURES

2.1. INTRODUCTION

The intent of this chapter is to provide background information relating to the diesel engine, the pollutants it emits, the history of its use in highway vehicles and railroad locomotives, diesel exhaust composition and emissions trends, and air pollution regulatory standards for diesel engines in the United States. The chapter also provides specific information about physical and chemical composition of diesel exhaust, descriptions of its atmospheric transformations, observations of measured and modeled ambient concentrations (considered alone and as a component of atmospheric particles in general), and some preliminary estimates of population exposures. This information provides background information that is used in conjunction with the toxicological and epidemiology data to formulate the conclusions about human health hazards that are discussed in later chapters of this document. The exposure information does not represent a formal or rigorous exposure assessment; it is only intended to provide a context for the health effects data and health hazard findings.

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 excellent fuel economy remains one of its strongest selling points. In the United States, the diesel engine is used mainly in trucks, buses, agricultural and other off-road equipment, locomotives, and ships.

The chief advantages of the diesel engine over the gasoline engine are its fuel economy and durability. Diesel engines, however, emit a higher mass of carbonaceous particulate matter than do gasoline engines. Over the past decade, modifications of diesel engine components have substantially reduced particle emissions (Hammerle et al., 1994; Sawyer and Johnson, 1995).

The diesel engine compresses air to high pressure and temperature. Fuel, when injected into this compressed air, autoignites, releasing its chemical energy. 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. 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 (also called compression-ignition, CI) 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/truck, small or large industrial, and rail or marine.

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. It must: (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 electronic components 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 is a mixture of many different hydrocarbon molecules from about C_7, to about C_{35}, with a boiling range from roughly 350 to 650°F. Many of the fuel and oil properties, such as its specific energy content (which is higher than gasoline), ignition quality, and specific gravity, are related to its hydrocarbon composition. Therefore, fuel and lubricant composition affects many aspects of engine performance, including fuel economy and exhaust emissions.

Complete and incomplete combustion of fuel in the diesel engine results in the formation of a complex mixture of gaseous and particulate exhaust. Because of concerns over health effects associated with diesel particulate emissions, EPA began regulating emissions from diesel engines in 1970 (for smoke) and then added regulations for gaseous emissions. EPA first regulated particulate emissions from HD diesels in 1988.

This chapter begins with background information regarding the formation of primary emissions resulting from diesel combustion, a summary of EPA emission standards for on-road and locomotive diesel engines, and a description of the national trends in emissions from on- and off-road diesel sources. The chapter continues with a description of engine technologies and the history of dieselization for on-road vehicles and locomotives, then provides a chronological assessment of emission rates and the chemical and physical nature of emissions. The data describing diesel engine emissions consider primary emissions, which undergo chemical and physical transformations in the atmosphere. Since the atmospheric transformations potentially have important impacts on environmental and human health, the available information regarding these transformations is discussed. This chapter concludes with a summary of the available literature regarding concentrations and exposures to diesel particulate matter (PM) in different exposure settings.

2.2. PRIMARY DIESEL EMISSIONS

2.2.1. Diesel Combustion and Formation of Primary Emissions

A basic understanding of diesel combustion processes can assist in understanding the complex factors that influence the formation of PM and other diesel exhaust emissions. Unlike spark-ignition combustion, diesel combustion is a fairly nonhomogenous process. Fuel is sprayed at high pressure into the compressed cylinder contents (primarily air with some residual combustion products) as the piston nears the top of the compression stroke. The turbulent mixing of fuel and air that takes place is enhanced by injection pressure, the orientation of the intake ports (e.g., inducement of intake-swirl tangential to the cylinder wall), piston motion, and piston bowl shape. In some cases fuel and air mixing is induced via injection of the fuel into a turbulence-generating pre-chamber or swirl chamber located adjacent to the main chamber (primarily in older, higher speed engines and some LD diesels). Examples of typical direct injection (DI) and indirect injection (IDI) combustion systems are compared in Figure 2-1. Diesel combustion can be considered to consist of the following phases (Heywood, 1988; Watson and Janota, 1982):

1. An ignition delay period, which starts after the initial injection of fuel and continues until the initiation of combustion. The delay period is governed by the rate of fuel and air mixing, diffusion, turbulence, heat transfer, chemical kinetics, and fuel vaporization. Fuel cetane rating is an indication of ignition delay.
2. Rapid, premixed burning of the fuel and air mixture from the ignition delay period.
3. Diffusion-controlled burning, in which the fuel burns as it is injected and diffuses into the cylinder.
4. A very small amount of rate-controlled burning during the expansion stroke, after the end of injection.

Engine speed and load are controlled by the quantity of fuel injected. Thus, the overall fuel-to-air ratio varies as engine speed and load vary. On a macro scale the cylinder contents are always fuel-lean. Depending on the time available for combustion and the proximity of oxygen, the fuel droplets are either completely or partially oxidized. At temperatures above 1300 K, unburned fuel that is not oxidized is pyrolized (stripped of hydrogen) to form elemental carbon soot (Dec and Espey, 1995). Soot formation occurs primarily during the diffusion-burn phase of combustion, and is highest during high load and other conditions consistent with high fuel-air

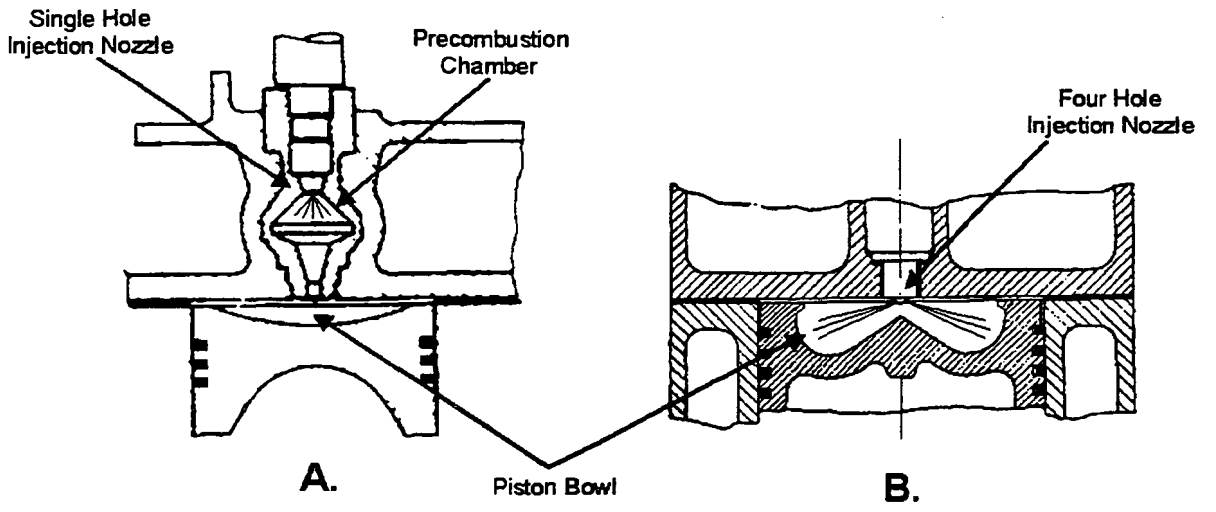

Figure 2-1. A comparison of IDI (A) and DI (B) combustion systems of high-speed, HD diesel truck engines. DI engines almost completely replaced IDI engines for these applications by the early 1980s.

equivalence ratios. Most of the soot formed (80% to 98%) is oxidized during later stages of combustion, most likely by hydroxyl (OH) radicals formed during combustion (Kittelson et al., 1986; Foster and Tree, 1994). The remainder of the soot leaves as a component of PM emissions from the engine.

During combustion, sulfur compounds present in the fuel are oxidized to sulfur dioxide (SO_2). Approximately 1% to 4% of fuel sulfur is oxidized to SO_3, which combines with water vapor in the exhaust to form sulfuric acid (H_2SO_4) (Wall et al., 1987; Khatri et al., 1978; Baranescu, 1988). Upon cooling, sulfuric acid and water condense into an aerosol that is nonvolatile under ambient conditions. The mass of sulfuric acid PM is more than doubled by the

mass of water associated with the sulfuric under typical PM measurement conditions (50% relative humidity, 20-25 °C) (Wall et al., 1987).

Oxide of nitrogen (NO_x) emissions from combustion engines, primarily (at least initially) in the form of NO, are generally thought to be formed via the Zeldovich mechanism, which is highly temperature dependent. High combustion temperatures cause reactions between oxygen and nitrogen to form NO and some NO_2. The majority of NO_2 formed during combustion is rapidly decomposed. NO can also decompose to N_2 and O_2, but the rate of decomposition is very slow because of the rapidly decreasing temperatures from the expansion of combustion gases during the expansion stroke (Heywood, 1988; Watson and Janota, 1982). Thus, most of the NO_x emitted is NO.

Some organic compounds from unburned fuel and from lubricating oil consumed by the engine can be trapped in crevices or cool spots within the cylinder and thus are not sufficiently available to conditions that would lead to their oxidation or pyrolysis. These compounds are emitted from the engine and either contribute to gas-phase organic emissions or to PM emissions, depending on their volatility. Within the exhaust system, temperatures are sufficiently high that these compounds are entirely present within the gas phase (Johnson and Kittelson, 1996). Upon cooling and mixing with ambient air in the exhaust plume, some of the less volatile organic compounds can adsorb to the surfaces of soot particles. Lacking sufficient soot adsorption sites, the organic compounds may condense on sulfuric acid nuclei (Abdul-Khalek et al., 1999).

Metallic compounds from engine component wear, and from compounds in the fuel and lubricant, contribute to PM mass. Ash from oil combustion also contributes trace amounts to PM mass.

2.2.2. Diesel Emission Standards and Emission Trends Inventory

EPA set a smoke standard for on-road HD diesel engines beginning with the 1970 model year, and then added a CO standard and a combined hydrocarbon (HC) and NO_x standard for the 1974 model year, as detailed in Table 2-1. Beginning in the 1979 model year, the EPA added a HC standard while retaining the combined HC and NO_x standard. All of the testing for HC, CO, and NO_x was completed using a steady state test procedure. Beginning in the 1985 model year, the EPA added a NO_x standard, dropped the combined HC and NO_x standard, and converted from steady-state to transient testing for HC, CO, and NO_x emissions. EPA introduced a particulate standard for the 1988 model year.

Since the 1985 model year, only the NO_x and particulate standards have been tightened for diesel engines. For truck and bus engines, the particulate standard was reduced in 1991, and again in 1994 for truck engines. For urban bus engines, the particulate standard was reduced in

Table 2-1. Emission standards: HD highway diesel engines

Model year	Pollutant (g/bhp-hr)					Smoke[a]
	HC	CO	NO_x	HC + NO_x	Particulate (PM) t=truck, b=bus, ub=urban bus	
1970	----	-----	-----	-----	----	A:40%; L:20%
1974	----	40	-----	16[b]	----	A:20%; L:15%; P:50%
1979	1.5	25	-----	10[b]	----	A:20%; L:15%; P:50%
1985[c]	1.3	15.5	10.7	----	----	A:20%; L:15%; P:50%
1988	1.3	15.5	10.7	----	0.60	A:20%; L:15%; P:50%
1990	1.3	15.5	6.0	----	0.60	A:20%; L:15%; P:50%
1991	1.3	15.5	5.0	----	0.25	A:20%; L:15%; P:50%
1993	1.3	15.5	5.0	----	0.25 t, 0.10 b	A:20%; L:15%; P:50%
1994	1.3	15.5	5.0	----	0.10 t, 0.07 ub	A:20%; L:15%; P:50%
1996	1.3	15.5	5.0	----	0.10 t, 0.05 ub	A:20%; L:15%; P:50%
1998	1.3	15.5	4.0	----	0.10 t, 0.05 ub	A:20%; L:15%; P:50%
2004	1.3	15.5	----	2.4 NMHC[d]	0.10 t, 0.05 ub	A:20%; L:15%; P:50%

[a]Emissions measured in percent opacity during different operating modes: A=Acceleration; L=Lug; P=Peaks during either mode.
[b]Total HC.
[c]In 1985, test cycle changed from steady-state to transient operation for HC, CO, and NO_x, measurement and in 1988 for PM.
[d]Or 2.5 plus a limit of 0.5 nonmethane hydrocarbon (NMHC).

1994 and again in 1996. The NO_x standard was reduced in 1998 for all on-road diesel engines, bus and truck. For 2004, the standards were further lowered in a 1997 rulemaking, with limits on non-methane hydrocarbon (NMHC) and NO_x combined, but no further reductions in CO, particulate matter, or smoke. These lower NMHC-plus-NO_x levels will very likely be confirmed in the "1999 technology review" of these standards. EPA is currently evaluating further reductions in NO_x and particulate matter for the post-2004 time frame.

In December 1997, the EPA adopted emission standards for NO_x, HC, CO, PM, and smoke for newly manufactured and remanufactured railroad locomotives and locomotive engines. The rulemaking, which takes effect in the year 2000, applies to locomotives originally manufactured from 1973, any time they are manufactured or remanufactured (locomotives originally manufactured before 1973 are not regulated). Three sets of emission standards have

manufactured from 1973 through 2001 (Tier 0), from 2002 through 2004 (Tier 1), and in 2005 and later (Tier 2) (Table 2-2; see EPA web page at http://www.epa.gov/omswww/ or http://www.dieselnet.com/standards/ for current information on mobile source emission standards). The emissions are measured over two steady-state test cycles which represent two different types of service, including the line-haul (long-distance transport) and switch (involved in all transfer and switching operations in switch yards) locomotives.

The EPA emission trends report (U.S. EPA, 1998a) provides emission inventories for criteria pollutants (PM10, PM2.5, SO_2, NO_x, VOC, CO, Pb, and NH_3) from point, area, and mobile sources, which indicate how emissions have changed from 1970 to 1977. For the purposes of this document, primary and secondary emissions from diesel engines (on- and off-road) are briefly discussed for PM10, sulfur dioxide (SO_2), nitrogen oxides (NO_x), and volatile organic compounds (VOC).

Mobile-source particulate emissions come from both gasoline- and diesel-powered engines in on-road vehicles and from a number of nonroad sources. Nonroad sources include aircraft, commercial boats (which are mainly diesel-powered), construction equipment, agricultural equipment, lawn/garden equipment, and other sources. The EPA emission trends report shows that among point, area, and mobile sources (excluding fugitive dust sources), mobile sources are responsible for 24% of PM10 emissions, with stationary sources (fuel combustion and industrial processes) responsible for the remainder.

Particulate emissions from diesels are much greater than those from gasoline-fueled engines. Particulate emissions (PM10) from gasoline-fueled engines decreased dramatically in 1975 with the widespread introduction of unleaded gasoline. Particulate emissions from diesel highway vehicles have decreased recently because of EPA emission standards for new model

Table 2-2. Emission standards: locomotives (g/bhp/hr)

	Year[a]	CO	HC	NO_x	PM
Line-haul	1973-2001 (Tier 0)	5.0	1.0	9.5	0.6
Switch	1973-2001 (Tier 0)	8.0	2.1	14.0	0.72
Line-haul	2002-2004 (Tier 1)	2.2	0.55	7.4	0.45
Switch	2002-2004 (Tier 1)	2.5	1.2	11.0	0.54
Line-haul	2005 + (Tier 2)	1.5	0.3	5.5	0.20
Switch	2005 + (Tier 2)	2.4	0.6	8.1	0.24

[a]Date of engine manufacture.

year HD diesel trucks that were first implemented in 1988 and became increasingly stringent in 1991 and 1994, as presented in Table 2-1 above.

The EPA emission trends report indicates that annual on-road vehicle PM10 emissions decreased from 397,000 tons to 268,000 tons from 1980 to 1997. Passenger car particulate emissions decreased from 120,000 to 56,000 tons (53%) in this time frame while diesel vehicle emissions decreased much less, from 208,000 to 163,000 tons (22%). Nonroad diesel engine particulate emissions decreased from 439,000 tons in 1980 to 316,000 tons in 1997 (28%). Emissions data for PM2.5 are available only for the period from 1990 to 1997, prohibiting an analysis of emission trends over the same time period as the other pollutants. For comparison to PM10, annual on-road diesel vehicle PM2.5 emissions were estimated at 144,000 tons in 1997 and nonroad diesel PM2.5 emissions in 1997 were 290,000 tons.

Diesel engines also contribute to secondary PM formation from NO_x and SO_2 emissions that are converted to nitrate and sulfate, although the direct emission of carbonaceous diesel particulates are much greater than secondary nitrate or sulfate formation. In 1997, about 50% of total ambient NO_x came from mobile sources, with diesels responsible for 26%, or approximately half of the mobile source contribution. About 6% of SO_2 came from mobile sources in 1997, with diesels responsible for 80% of that total. VOC emissions from diesel engines in 1997 were estimated at 4% of the total emissions from all sources.

2.2.3. Engine Technology Description and Chronology

NO_x emissions, PM emissions, and brake-specific fuel consumption (BSFC) are among the parameters that are typically considered during the development of a diesel engine. Many engine variables that decrease NO_x can also increase PM and BSFC. One manifestation of the interplay among NO_x, PM, and BSFC is that an increase in combustion temperatures will tend to increase NO formation via the Zeldovich mechanism, will often improve thermal efficiency, can improve BSFC, and can increase the rate of PM oxidation, thus lowering PM emissions. One example of this is the tradeoff of PM emissions and BSFC versus NO_x emissions with fuel injection timing. Many recent advances in reducing the engine-out emissions of diesel engines are combinations of technologies that provide incremental improvements in the tradeoffs among these different emissions and fuel consumption. The sum total, though, can be considerable reductions in regulated emissions within acceptable levels of fuel consumption.

The majority of current HD diesel truck engines certified for use in the United States utilize:

- a 4-stroke cycle;
- direct-injection, high-pressure (1200 bar to >2000 bar) fuel injection systems with electronic control of injection timing and, in some cases, injection rate;
- centrally located multihole injection nozzles;
- 3 or 4 valves per cylinder;
- turbochargers;
- in many cases, air-to-air aftercooling; and
- in some cases, the use of an oxidation catalyst.

These features have phased into use with HD truck engines because they offer a relatively good combination of fuel consumption, torque-rise, emissions, durability, and the ability to better "tune" the engines for specific types of applications. Fuel consumption, torque-rise, and drivability have been maintained or improved while emissions regulations have become more stringent. Many Class 8a and 8b diesel truck engines are now capable of 700,000 to 1,000,000 miles of driving before their first rebuild, and can be rebuilt several times because of their heavy construction and the use of removable cylinder liners. This is several times the regulatory estimate of full useful life for HD engines (290,000 miles) previously used by EPA.

Current 4-stroke locomotive engines use engine technology similar to on-highway diesel engines, except that electronic controls have only recently been introduced. It is difficult to separate the components of current high-speed diesel engines for discussion of their individual emissions effects. Most of the components interact in numerous ways that affect emissions, performance, and fuel consumption.

2.2.3.1. *Injection Rate*

Decreasing the duration of diffusion combustion and promoting soot oxidation during the expansion stroke can reduce formation of soot agglomerates (Stone, 1995). Both of these effects are enhanced by increasing the fuel injection rate. The primary means of accomplishing this is by increasing fuel injection pressure. Increased injection rate can significantly reduce soot emissions, but it can also increase combustion temperatures and cause an increase in NO_x emissions (Springer, 1979; Watson and Janota, 1982; Stone, 1995). However, when combined with turbocharging, aftercooling, and injection timing retard, low NO_x, low PM, and relatively good BSFC and brake mean engine pressure (BMEP) are possible.

In 1977 Robert Bosch introduced a new type of high-pressure pump capable of producing injection pressures of 1700 bar at the nozzle (Voss et al., 1977). This increased fuel injection pressure by roughly a factor of 10. Unit injection, which combines each fuel injection nozzle

with individual cam-driven fuel pumps, can achieve very high injection pressures (>2000 bar). The first combination of unit injectors with electronically controlled solenoids for timing control was offered in the United States by Detroit Diesel Corporation in the 1988 model year (Hames et al., 1985). Replacement of the injection cam with hydraulic pressure, allowing a degree of injection rate control, was made possible with the hydraulic-electronic unit injection (HEUI) jointly developed by Caterpillar and Navistar, introduced on the Navistar T444E engine (and variants) in 1993.

It is widely known that high fuel injection pressures have been used to obtain compliance with the PM standards that went into effect in 1988 (Zelenka et al., 1990). Thus, it is likely that a transition to this technology began in the 1980s, with the vast majority of new engine sales employing this technology by 1991, when the 0.25 g/bhp-h Federal PM standard went into effect.

The use of electronic control of injection rate is rapidly increasing on medium-HD diesel engines equipped with HEUI (currently available on Caterpillar 3126 and Navistar T444E, DT466, and 530E engines). Engines are currently under development, perhaps for 2002-2004 introduction, that use common-rail fuel injection systems with even more flexible control over injection pressure and timing than previous systems.

2.2.3.2. *Turbocharging, Charge-Air Cooling, and Electronic Controls*

Use of exhaust-driven turbochargers to increase intake manifold pressure has been applied to both IDI and DI diesel engines for more than 40 years. Turbocharging can decrease fuel consumption compared to a naturally aspirated engine of the same power output. Turbocharging utilizes otherwise wasted exhaust heat to generate intake boost. The boosted intake pressure effectively increases air displacement and increases the amount of fuel that can be injected to achieve a given fuel-air equivalence ratio. Turbocharging increases the power density of an engine. Boosting intake pressure via turbocharging and reducing fuel-to-air ratio at a constant power can significantly increase both intake temperatures and NO_x emissions. Increased boost pressure can significantly reduce ignition delay, which reduces VOC and PM soluble organic fraction (SOF) emissions (Stone, 1995) and increases the flexibility in selection of injection timing. Injection timing on turbocharged engines can be retarded further for NO_x emission control with less of an effect on PM emissions and fuel consumption. This allows a rough parity in NO_x emissions between turbocharged (non-aftercooled) and naturally aspirated diesel engines (Watson and Janota, 1982).

Turbocharging permits the use of higher initial injection rates (higher injection pressure), which can reduce particulate emissions. Although this may offer advantages for steady-state operation, hard accelerations can temporarily cause overly fuel-rich conditions because the

turbocharger speed lags behind a rapid change in engine speed (turbo-lag). This can cause significant increases in PM emissions during accelerations. Before the advent of electronic controls, the effect of acceleration on PM emissions could be limited by mechanically delaying demand for maximum fuel rate with a "smoke-puff eliminator." Since this device also limited engine response, there was considerable incentive for the end-users to remove or otherwise render the device inactive. Charge-air cooling, for example using an air-to-air aftercooler (air-cooled heat exchanger) between the turbocharger compressor and the intake manifold, can greatly reduce intake air and peak combustion temperatures. When combined with injection timing retard, charge-air cooling allows a significant reduction in NO_x emissions with acceptable BSFC and PM emissions when compared to either non-aftercooled or naturally aspirated diesel engines (Hardenberg and Fraenkle, 1978; Pischinger and Cartellieri, 1972; Stone, 1995) (Figure 2-2).

Electronic control of fuel injection timing allowed engine manufacturers to carefully tailor the start and length of the fuel injection events much more precisely than through mechanical means. Because of this, newer on-highway turbocharged truck engines have virtually no visible smoke on acceleration. Electronic controls also allowed fuel injection retard under desirable conditions for NO_x reduction, while still allowing timing optimization for reduced VOC emissions on start-up, acceptable cold-weather performance, and acceptable performance and durability at high altitudes. Previous mechanical unit injected engines (e.g., the 1980s Cummins L10, the non-DDEC DDC 6V92) were capable of reasonably high injection

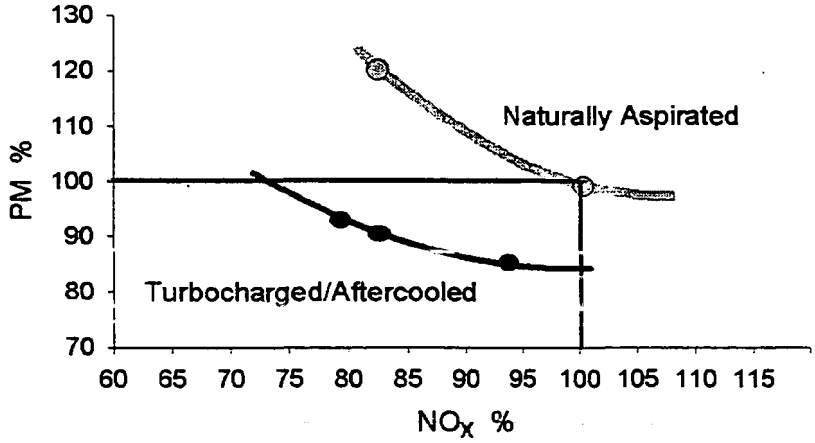

Figure 2-2. Effect of turbocharging and aftercooling on NO_x and PM (Mori, 1997).

pressures, but had fixed injection timing that only varied based on the hydraulic parameters of the fuel system. Many other engines with mechanical in-line or rotary injection pumps had only coarse injection timing control or fixed injection timing.

Precise electronic control of injection timing over differing operating conditions also allowed HD engine manufacturers to retard injection timing for low NO_x emissions during highly transient urban operation similar to that found during emissions certification, and advance the injection timing during less transient operation (such as freeway driving) for fuel consumption improvements (~3% to 5%) at the expense of greatly increased NO_x emissions (~3 to 4 times regulated levels). This particular situation resulted in the recent consent-decree settlements between the Federal Government and most of the HD engine manufacturers to assure effective NO_x control in all driving conditions.

Turbocharged engines entered the market very slowly beginning in the 1960s. During the years 1949 to 1975 the total improvement in emissions for the on-road diesel fleet was considerably less than 10%-20% for gaseous emissions and, for particulates, there was really no change at all until the advent of particulate standards in 1988. Charge air cooling was introduced during the 1960s and was initially performed in a heat exchanger using engine coolant. Cooling of the charge air using ambient air as the coolant was introduced by Mack in 1977 with production of the ETAY(B)673A engine (Heywood, 1988). Use of ambient air allowed cooling of the charge air to much lower temperatures. Most HD diesel engines sold today employ some form of charge air cooling, with air-to-air aftercooling the most common. Johnson and co-workers (Johnson et al., 1994) have presented a comparison of similar engines that differ in that the charge air is cooled by engine coolant (1988 engine) and by ambient air with a higher boost pressure for the second (1991 engine). The 1991 engine also used higher pressure fuel injectors. The 1991 engine exhibited both lower PM (50%) and NO_x emissions. Higher injection pressure likely enabled the reduced PM emissions, while the lower charge air temperature and the ability to electronically retard the injection timing under some conditions likely enabled the lower NO_x emissions.

It is apparent on the basis of both the literature and certification data that turbochargers with aftercoolers can be used in HD engines in conjunction with other changes to result in a decrease in emissions. NO_x was probably reduced on the order of 10% to 30% in turbocharged aftercooled engines with retarded injection timing. Prior to the late 1970s, only a portion of all HD diesel engines were turbocharged, so the total improvement in emissions that could be associated with these changes was considerably less until more stringent emissions regulations were implemented. The lowest combination of in-use NO_x and PM emissions would likely be for turbocharged aftercooled engines that used retarded, high-pressure unit injection without

electronic control in the early 1990s. Although tighter NO_x standards phased in for model years 1994 and 1998, this is complicated by the instances of defeating NO_x control during cruise conditions by most engine manufacturers. Defeat of NO_x control occurred to a different extent with all Class 8 electronically controlled engines beginning with their introduction in 1988. PM emissions were likely much lower for engines on which electronic controls were introduced, but NO_x emissions in-use were likely much higher than for early electronic or late mechanically injected versions of the engines. Overall, it is expected that engines in the 1950 to 1980 time frame would have PM emissions similar to those of the mid-1980 engines that were not yet controlled for particulates, while later engines would have lower PM emissions.

2.2.3.3. *Indirect and Direct Injection High-Speed Diesel Engines*

Prior to the 1930s, diesel engine design was limited to relatively low-speed applications because sufficiently high-pressure fuel injection equipment was not available. With the advent of high-speed and higher pressure pump-line-nozzle systems, introduced by Robert Bosch in the 1930s, it became possible to inject the fuel directly into the cylinder for the first time, although IDI diesel engines continued in use for many years. As diesels were introduced into the heavy truck fleet in the 1930s through the 1950s, both IDI and DI naturally aspirated variants were evident. A very low-cost, rotary injection pump technology was introduced by Roosa-Master in the 1950s, reducing the cost of DI systems and allowing their introduction on smaller displacement, higher speed truck engines.

DI diesel engines have now all but replaced IDI diesel engines for HD on-highway applications[1]. IDI engines typically required much more complicated cylinder head designs, but generally were capable of using less sophisticated, lower pressure injection systems with less expensive single-hole injection nozzles. IDI combustion systems are also more tolerant of lower grades of diesel fuel. Fuel injection systems are likely the single most expensive component of many diesel engines. Caterpillar continued producing both turbocharged and naturally aspirated IDI diesel engines for some on-highway applications into the 1980s. Caterpillar and Deutz still produce engines of this type, primarily for use in underground mining applications. IDI combustion systems are still used in many small-displacement (<0.5 L/cylinder), very high-speed (>3000 rpm rated speed) diesel engines for small offroad equipment (small imported tractors, skid-steer loaders), auxiliary engines, and small generator sets.

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[1]The GM Powertrain/AM General 6.5L electronically controlled, turbocharged IDI-swirlchamber engine, certified as a light-HD diesel truck engine, is the last remaining HD on-highway IDI engine sold in the United States.

IDI engines have practically no premixed burn combustion, and thus are often quieter and have somewhat lower NO_x emissions than DI engines. Electronic controls, high-pressure injection (e.g., GM 6.5), and 4-valve/cylinder designs (e.g., the 6-cylinder Daimler LD engine) can be equally applied to IDI diesel engines as with their DI counterparts, but negate any advantages in cost over DI engines. DI diesel engines of the same power output consume 15%-20% less fuel than IDI engines (Heywood, 1988). Considering the sensitivity of the HD truck market to fuel costs, this factor alone likely accounts for the demise of IDI diesel engines in these types of applications. Throttling and convective heat transfer through the chamber-connecting orifice, and heat rejection from the increased surface area of IDI combustion systems, decreases their efficiency and can cause cold-start difficulties when compared to DI designs. Most IDI diesel engine designs require considerably higher than optimum (from an efficiency standpoint) compression ratios to aid in cold starting (19:1 to 21:1 versus ~15:1 to 17:1 for DI engines).

Because of the early introduction of DI technology into truck fleets, it is likely that by end of the 1970s, only a small fraction of the HD diesel engines sold for on-highway use were IDI engines. It is unlikely that the gradual shift from IDI to DI engine designs through the 1960s and 1970s had any significant impact on emissions.

2.2.3.4. *Two-Stroke and 4-Stroke High-Speed Diesel Engines*

A detailed discussion of the 2- and 4-stroke engine cycles can be found in Heywood, Taylor, or Stone, and so will not be presented here (Heywood, 1988; Taylor, 1990; Stone, 1995). Nearly all high-speed 2-stroke diesel engines utilize uniflow scavenging assisted by a positive displacement blower (Figure 2-3). Uniflow-scavenged 2-stroke diesels use poppet exhaust valves similar to those found in 4-stroke engines. The intake air enters the cylinder through a pressurized port in the cylinder wall. A crankshaft-driven, positive-displacement blower (usually a roots-type) pressurizes the intake port to ensure proper scavenging. A turbocharger may be added to the system to provide additional boost upstream of the blower at higher speeds, and to reduce the size and parasitic losses associated with the positive-displacement blower.

Two-stroke diesel engines can achieve efficiency comparable to 4-stroke counterparts and have higher BMEP (torque per unit displacement) (Heywood, 1988). It is useful to note that the 2-stroke cycle fires each cylinder once every revolution, while the 4-stroke cycle fires every other revolution. Thus, for a given engine size and weight, 2-strokes can produce more power. However, 2-stroke diesel engines are less durable than their 4-stroke counterparts. Lubricating oil is transferred from the piston rings to the intake port, which causes relatively high oil

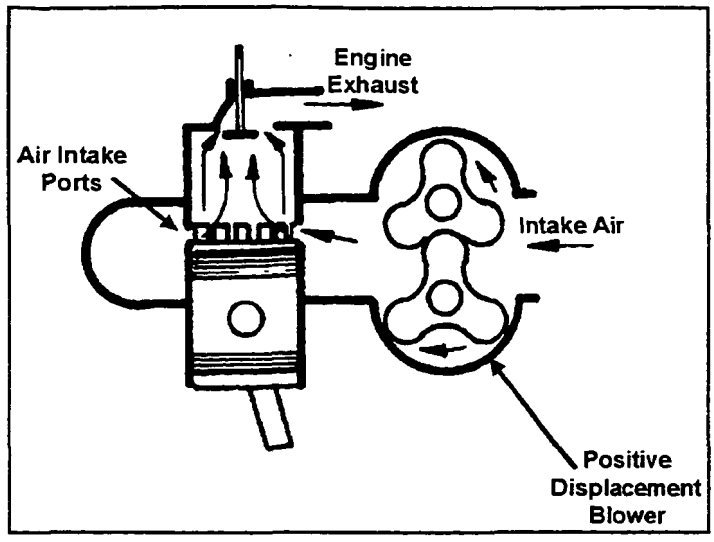


Figure 2-3. An example of uniflow scavenging of a 2-stroke diesel engine with a positive displacement blower (Adapted from Taylor, 1990). Scavenging is the process of simultaneously emptying the cylinder of exhaust and refilling with fresh air.

consumption relative to 2-stroke designs. Durability and low oil consumption are desirable for on-highway truck applications. This may be why 4-stroke engines have been favored for these applications since the beginning of dieselization in the trucking industry, with the notable exception of urban bus applications. Although it is no longer in production, the Detroit Diesel 6V92 series of 2-stroke diesel engines is still the most popular for urban bus applications, where the high power density allows the engine to be more easily packaged within space limitations. The primary reason that 2-stroke engines like the 6V92 are no longer offered for urban bus applications is PM emissions. The reduced lubricating oil control with 2-strokes tends to increase VOC and organic PM emissions relative to 4-stroke designs. This was particularly problematic for urban bus applications because urban bus engines must meet tighter Federal and California PM emissions standards. The current urban bus PM standard (0.05 g/bhp-hr) is half of the current on-highway HD diesel engine PM standard. No 2-stroke diesel engine designs have been certified to meet the most recent urban bus PM emissions standards, and Detroit Diesel Corporation has not certified a 2-stroke diesel engine for on-highway truck use since 1995.

A comprehensive review of emissions from hundreds of later model vehicles (1976-1998) found no significant difference between 2- and 4-stroke vehicles (Yanowitz et al., 1999a). Overall, regulated emissions changes due to changing proportions of 2- and 4-stroke engines in the in-use fleet during the years 1949-1975 do not appear to be significant for HD truck and bus

engines. Furthermore, it appears that the proportion of 2-stroke engines in the in-use fleet was relatively constant until the 1980s.

2.2.3.5. *Near-Term Diesel Emission Reduction Technologies*

2.2.3.5.1. *Exhaust gas recirculation.* Exhaust gas recirculation (EGR) (i.e., routing some of the exhaust gas to the intake manifold) is widely used in LD SI gasoline engines to control NO_x emissions. Unlike most SI applications, use of EGR with diesels necessitates both electronic control of the EGR as well as EGR cooling to limit the associated increase in PM. Because EGR displaces part of the intake air, it can increase the overall fuel-to-air equivalence ratio to a point that can lead to large increases in PM emissions. Hot EGR further exacerbates this problem by increasing the temperature of the intake air. The increased temperature decreases air density and further reduces the volume of intake air entering the engine.

Cooled EGR systems typically use an engine coolant heat exchanger to cool the recirculated exhaust gases before mixing with the intake air. EGR cooling has the potential to significantly reduce the increase in intake air temperature associated with EGR. This would mitigate (though not eliminate) the PM emissions penalty associated with diesel EGR systems. Though EGR cooling greatly extends the operational range over which EGR can be used, electronic control of the EGR will be necessary to prevent large PM increases under hard acceleration, near peak torque conditions, or at high altitudes. EGR cooling can also reduce combustion temperatures beyond uncooled EGR, resulting in further decreases in NO_x emissions relative to uncooled EGR under certain conditions (Kakoi et al., 1998; Leet et al., 1998).

Cooled EGR is currently used with the relatively small number of LD diesel vehicles sold for the U.S. market. Although it is not widely used in HD diesel engines today, many believe that cooled EGR will be an important technology for future NO_x reductions (Johnson et al., 1994; Zelenka, 1990). Most, if not all, of the diesel engines that will meet either the 2002 consent decree requirements (early compliance with 2004 standards) or the 2004 emissions standards for HD trucks will incorporate some form of cooled EGR into their engine designs to meet the 2.5 g/bhp-hr NO_x + NMHC standard.

Ladomatos et al. (1996-1997) have described the three mechanisms by which EGR is thought to lead to reduced emissions of NO_x:

- *Dilution*: Recirculating exhaust gas leads to a reduction in the oxygen content of the intake charge. Although this increases ignition delay, it also reduces peak temperature. With respect to NO formation, the increased ignition delay and premixed burn fraction are more than offset by the dilution effects on peak

temperature, resulting in a significant reduction in NO_x emissions. The reduction in oxygen content can also cause an increase in HC, CO, and PM emissions. The dilution mechanism is thought to be, by far, the most important mechanism affecting diesel engine emissions.

- *Thermal*: The recirculated exhaust gas contains CO_2 and water vapor, which increase the specific heat of the intake charge. This lowers peak temperature and hence formation of NO_x is decreased.
- *Chemical*: It is possible that endothermic dissociation of recirculated CO_2 and water lowers peak temperature, leading to a reduction in formation of NO_x.

Early studies of uncooled diesel EGR were conducted by Pischinger and Cartellier (1972) and Springer (1979). Although sub-4 g/bhp-hr NO_x emissions levels were possible, the high PM emissions associated with the NO_x reductions delayed the introduction of EGR until fuel sulfur levels were low enough to enable engine-coolant cooling of EGR. Theoretically, further cooling of the EGR (for example, air cooling) would extend the range of engine operating conditions under which EGR could be used, but is not possible at current fuel sulfur levels because of the potential for very high levels of sulfuric acid condensation in the EGR cooler (McKinley, 1997; Kreso et al., 1998a; Leet et al., 1998).

Johnson (1994) noted that engine durability is a serious concern with EGR because recirculation of soot through the engine can increase wear. Kreso and co-workers (Kreso et al., 1998b) recently examined a 1995 Cummins M-11 at two steady-state modes and two EGR rates. The EGR system included cooling of the recirculated gas. EGR was effective at reducing NO_x, and reductions as high as 56% were observed under some conditions. Emissions of PM increased by as much as 57%, while emissions of the SOF portion of PM were somewhat lower. Examination of the mutagenicity of SOF with the Ames assay indicated that the SOF produced by EGR was more mutagenic.

It is clear that any EGR system will require careful control so that EGR is applied only under operating conditions where significant NO_x reductions can be obtained without a major increase in PM. There is little evidence to suggest that the character of PM, SOF, or gaseous hydrocarbon emissions is dramatically altered by use of EGR.

2.2.3.5.2. ***Diesel oxidation catalysts (DOC).*** DOCs for HD diesel applications were originally developed for underground mining equipment for exhaust odor and CO control (typically not issues for diesel engines outside of confined environments). The use of early high platinum-content DOCs was an issue for these applications because of their high levels of NO to NO_2 and

SO_2 to SO_3 oxidation (McClure, 1992). McDonald et al. (1995) found that the SO_3 oxidation rate was sufficient to produce ~0.2 g/bhp-hr sulfate PM emissions at high load conditions even with a relatively low-sulfur diesel fuel (0.01% S).

Later DOCs were developed that relied more on base metals and less on precious metals for VOC oxidation (lowering SOF PM) while limiting high-temperature formation of sulfuric acid PM. These types of catalysts were first applied to LD diesel vehicles in the 1980s, some urban bus applications (1994 Cummins L10), and a number of medium-HD diesel engines after 1993 (Navistar T444E, some versions of the Caterpillar 3116 and 3126). There are also a number of DOCs that are now being retrofitted to older urban buses as part of the EPA Urban Bus Retrofit and Rebuild Program. Current DOCs oxidize more than 70% of the VOCs that contribute to SOF PM, leading to a 15%-30% reduction in total PM emissions (Farrauto et al., 1996; Brown and Rideout, 1996; Tamanouchi et al., 1998).

DOCs are highly effective at oxidizing lube oil components (Farrauto et al., 1996) as well as most PAHs (Mitchell et al., 1994; Pataky et al., 1994; Bagley et al., 1996; McDonald, 1997; Bagley et al., 1998). There are conflicting data as to whether DOCs catalyze the formation or oxidation of nitro-PAH compounds. Bagley and co-workers (Bagley et al., 1998) and McDonald (1997) found reductions in both PAHs and nitro-PAH and associated mutagenic activity for a low-sulfate-forming base-metal/Pt/Pd oxidation catalyst that were statistically significant at $p<.01$, and found only one nitro-PAH (1-nitropyrene) above minimum detection limits in either catalyzed or uncatalyzed exhaust . Mitchell and co-workers (1994) found decreases in PAHs with twofold increases in nitro-PAH (statistical significance is not known). More comprehensive testing will be necessary to draw further conclusions about the effects of DOCs on nitro-PAH.

2.2.3.6. *Future (2004+) Diesel Emission Reduction Technologies*

2.2.3.6.1. *NO_x storage catalysts.* NO_x storage catalysts currently under development might be used to meet 2007 HD diesel engine standards if diesel fuel sulfur levels are considerably reduced (0-30 ppm S fuel may be necessary). A generalized schematic of the operation of this device is included in Figure 2-4. This catalyst system employs a high-platinum (Pt) content catalyst for oxidation of NO to NO_2 (in the absence of an oxidation catalyst, total NO_x in diesel exhaust is primarily NO [typically >90%] with lesser amounts of NO_2). The NO_2 is then stored, using one of a number of barium compounds, as barium nitrate. For approximately 2-second durations every 2 minutes, diesel fuel is either sprayed into the exhaust or injected into the cylinder after combustion to provide the necessary hydrocarbons to remove the NO_x from the storage components. The NO_x is then reduced over a standard three-way catalytic converter.

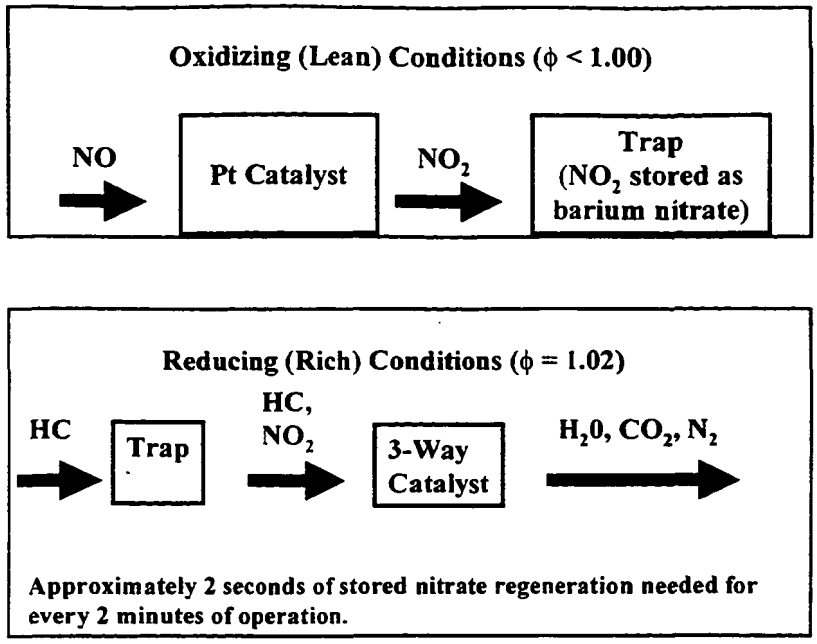


Figure 2-4. NO_x-storage catalyst operation under oxidizing and reducing conditions.

The average NO_x reduction potential for this technology over the FTP is 50% to 75%, with a fuel consumption penalty of approximately 3% to 5% (Wall, 1998). Figure 2-5 compares the NO_x reducing capabilities of a NO_x storage catalyst system to a representative sulfur-tolerant NO_x catalyst system.

2.2.3.6.2. ***Lean-NO_x catalysts.*** Various types of active (requiring a post-combustion fuel injection event) and nonactive (no post-injection) lean-NO_x catalysts are in production or are under investigation for continuous reduction of NO_x emissions in lean exhaust environments such as those present in diesel exhaust. (These are continuous devices, as opposed to the cyclic nature of NO_x reduction using NO_x storage catalysts.) Lean-NO_x catalysts typically reduce NO_x efficiently over a very narrow range of exhaust temperatures. There are both high- and low-temperature varieties of lean-NO_x catalysts. Low-temperature, platinum-based lean-NO_x catalysts using zeolites for support, catalyst promotion, and adsorption of NO_x and HC would be typical of a lean-NO_x catalyst technology for medium and light-HD diesel applications. High-temperature base-metal lean-NO_x catalyst formulations (Cu-ZSM, for example) are under investigation primarily for highly loaded HD diesel engine applications.

A number of new common-rail fuel injection systems are capable of injecting fuel after combustion to provide additional hydrocarbons for use as an NO_x reductant with lean-NO_x

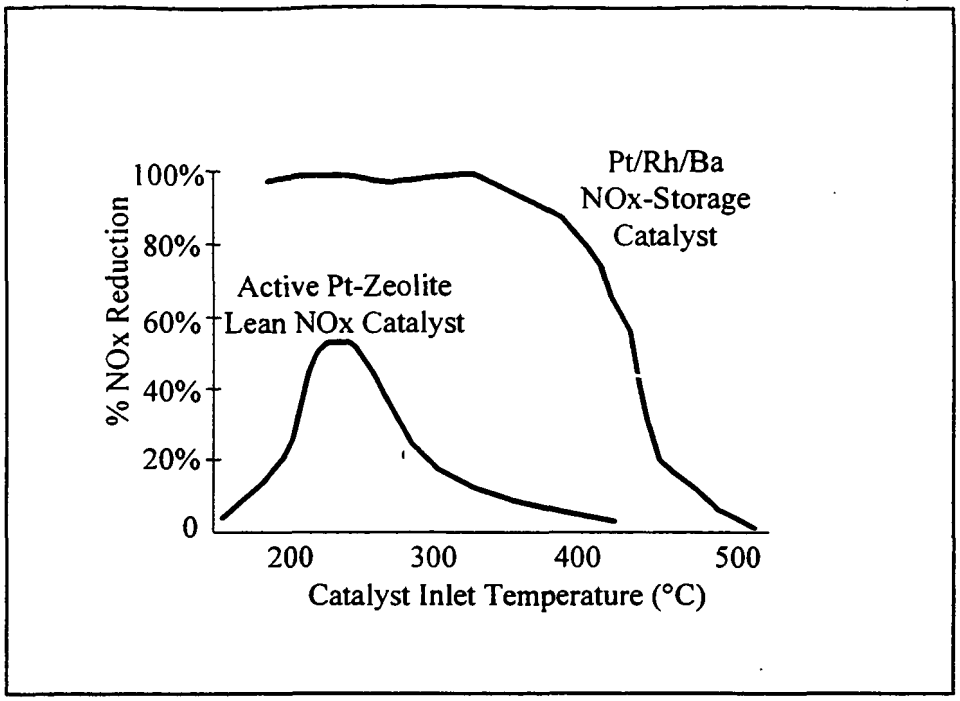


Figure 2-5. A comparison of the NO_x reduction efficiency over a range of temperature conditions for the sulfur-intolerant NO_x storage catalyst system and the more sulfur-tolerant, active Pt-zeolite catalyst system. Although peak NO_x reduction efficiencies for various types of nonstorage lean-NO_x catalysts (similar to the Pt-Zeolite catalyst shown here) approach 50%-60%, average reductions are 15% to 30% over various (FTP-75, NEDC) driving cycles.

catalysts. Although active Pt-zeolite catalyst systems have higher NO_x removal efficiencies than similar nonactive catalyst systems, NO_x removal efficiencies are still only in the range of 15% to 35% over the New European Drive Cycle (NEDC) (Peters et al., 1998; Engler et al., 1998) and significantly below those of NO_x storage catalyst systems (Figure 2-5). Newer systems use a controlled fuel-exotherm over the platinum catalysts with feedback control to maintain a more constant catalyst temperature, enabling higher NO_x reduction efficiencies.

2.2.3.6.3. ***Selective catalytic reduction.*** Selective catalytic reduction (SCR) for NO_x control is currently available for stationary diesel engines, and has been proposed for mobile light- and heavy-diesel applications. SCR uses ammonia as a reducing agent for NO_x over a catalyst composed of precious metals, base metals, and zeolite. The ammonia is supplied by introducing

a urea/water mixture into the exhaust upstream of the catalyst. The urea/water mixture is typically stored in a separate tank that must be periodically replenished. Ammonia has extremely high selectivity as a reductant for NO_x. NO_x reductions of 70% to 90% over a broad range of operating conditions are possible using such systems (Brown, 1998). These systems appear to be tolerant of current U.S. on-highway diesel fuel sulfur levels for exhaust temperatures that are consistent with heavier (Class 7, 8) HD on-highway applications and over the HD FTP test cycle (40 CFR, Subpart N). NO_x reduction efficiency drops considerably at exhaust temperatures less than 200°C in the presence of SO_2 in the exhaust. Therefore, the practical fuel sulfur limit for LD diesel applications is probably somewhat less than 100 ppm. This reduced efficiency at low temperatures and higher fuel sulfur levels may also have implications for "not-to-exceed" NO_x requirements for HD on-highway diesel engines introduced in the consent decrees and likely to be a component of both the 2004 and 2007 HD diesel emissions standards.

Control of the quantity of urea injection into the exhaust, particularly during transient operation, is an important issue with SCR systems. Injection of too large of a quantity of urea leads to a condition of "ammonia slip," whereby excess ammonia formation can lead to both direct ammonia emissions and oxidation of ammonia to produce (rather than reduce) NO_x. There are also a number of potential hurdles to overcome with respect to using a major emission control system that requires frequent replenishing of a consumable fluid in order to function. This raises issues related to supply, tampering, and the possibility of running the urea storage tank dry. Packaging of the urea supply within the constraints of modern LD vehicles may also be particularly challenging. Packaging of SCR systems does not appear to be a major problem for HD truck or bus applications.

2.2.3.6.4. ***Continuously regenerating traps.*** One method of exhaust aftertreatment for controlling diesel PM emissions is to pass diesel exhaust through a ceramic or metallic filter or "PM trap" to collect the PM, and to use some means of burning the collected PM so that the trap can be either periodically or continuously regenerated. Previous traps have used catalyzed coatings, fuel additives, and electrical heating to assist trap regeneration. Failure to consistently regenerate the trap can lead to plugging, excessive exhaust back-pressure, and eventually overheating and permanent damage to the trap. Inconsistent regeneration due to the high frequency of fairly low exhaust temperatures has been a particular problem in applying PM traps to some lightly loaded diesel applications.

The recently developed continuously regenerating trap (CRT) has shown considerable promise in a broad range of diesel applications because of its ability to regenerate even at fairly low exhaust temperatures. The CRT uses nitrogen dioxide (NO_2) to assist trap regeneration.

NO_2 can oxidize soot collected within the trap at exhaust temperatures as low as 250°C (Hawker et al., 1997), which is within the typical exhaust temperature range of many diesel vehicle and truck applications (Lüders et al., 1997). The NO_2 is produced by oxidizing NO in the exhaust using a high-platinum-content oxidation catalyst brick located immediately upstream of the ceramic trap-filter. A general schematic of the CRT system is presented in Figure 2-6.

The CRT is capable of reducing PM emissions by more than 80% (Hawker et al., 1997; Hawker et al., 1998). SO_2 inhibition of NO oxidation effectively limits the CRT to use with diesel fuel sulfur levels below 50 ppm.

In some cases, excess fuel can be used to induce an exotherm over the Pt-catalyst to ensure that minimum soot oxidation temperatures are reached.

It appears likely that introduction of emission standards that would force the use of CRT or similar catalyst/trap technologies would likely be accompanied by steep reductions in toxic emissions. Hawker and co-workers found substantial reductions in gas- and PM-phase VOC, soot, acetaldehyde, formaldehyde, and total particle number (Hawker, 1998). Considering that the CRT incorporates a Pt-DOC, PAHs oxidation is probably also high, but this was not determined in the study.

2.2.3.6.5. ***Possible effects of advanced aftertreatment systems.*** *NO_2 formation*: One constant among many of the various proposed diesel exhaust aftertreatment devices is the reliance on high Pt content for some components. In some cases, lean reactions of NO to NO_2 are integral to the

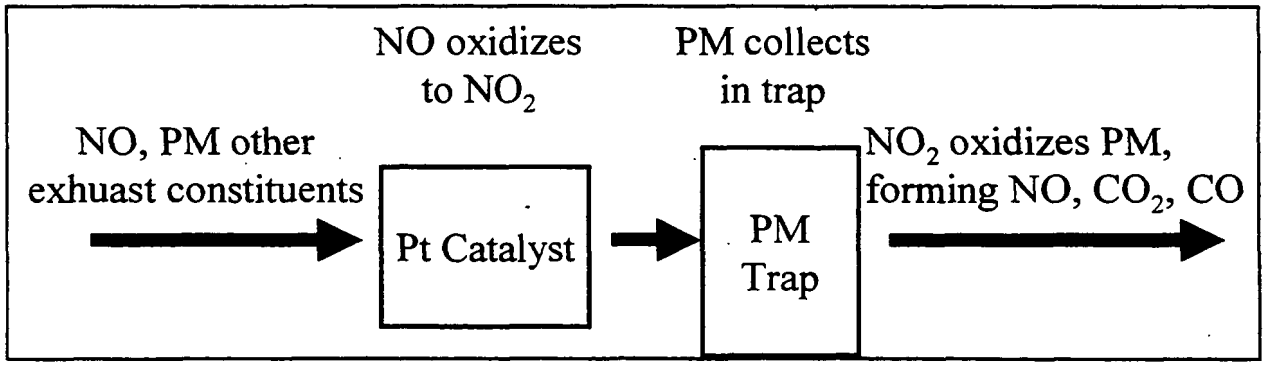


Figure 2-6. Schematic showing the operating principles of the continuously regenerating trap (CRT).

design of the device (CRT, NO_x storage catalyst). In the case of the CRT, >50% conversion of NO to NO_2 is desired for efficient trap regeneration (Figure 2-7). This could result in a significant increase in direct NO_2 emissions from diesel exhaust. In the case of NO_x storage catalysts and SCR, there may be less cause for concern because of the relatively high NO_x reduction efficiencies. Low-temperature lean-NO_x catalysts have relatively high Pt contents, but no data were found in the literature that quantified NO/NO_2 emissions for these devices.

Sulfate PM: The relatively high conversion rates of fuel sulfur to sulfuric acid aerosol possible with high-Pt content diesel exhaust aftertreatment systems are similar to those found with early high-Pt DOCs (for example, a Pt lean-NO_x catalyst in Figure 2-8), although it is likely that broad introduction of advanced diesel exhaust aftertreatment systems through reductions in standards for regulated emissions would be accompanied by significant fuel sulfur control.

Ammonia: Widespread use of urea-SCR catalyst systems could increase ammonia emissions. Newer SCR designs are incorporating electronic control of urea injection and the use of a "cleanup" catalyst for oxidation of excess ammonia to minimize ammonia emissions.

PM and VOC: Most of the aftertreatment systems under development are still too new to have been subjected to comprehensive exhaust speciation analyses. The CRT has the additional problem that PM emissions are so low that it is difficult to collect a large enough PM sample for

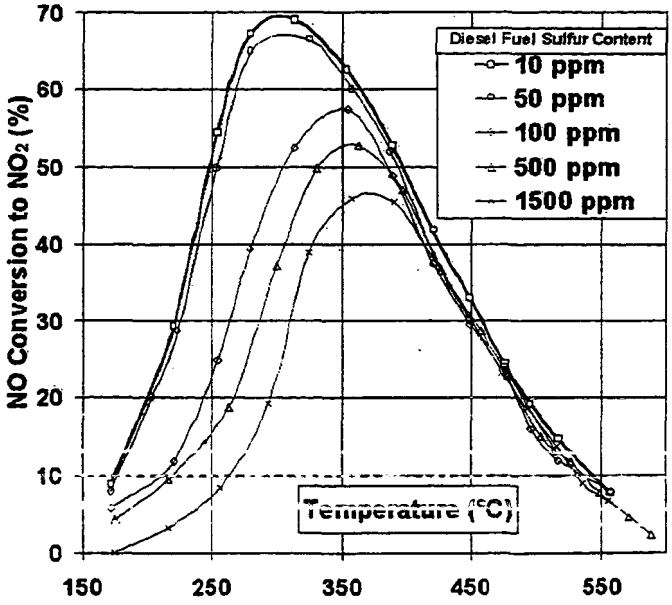


Figure 2-7. Efficiency of NO to NO_2 conversion over the oxidation catalyst component of the CRT at different exhaust temperatures and at differing diesel fuel sulfur levels.

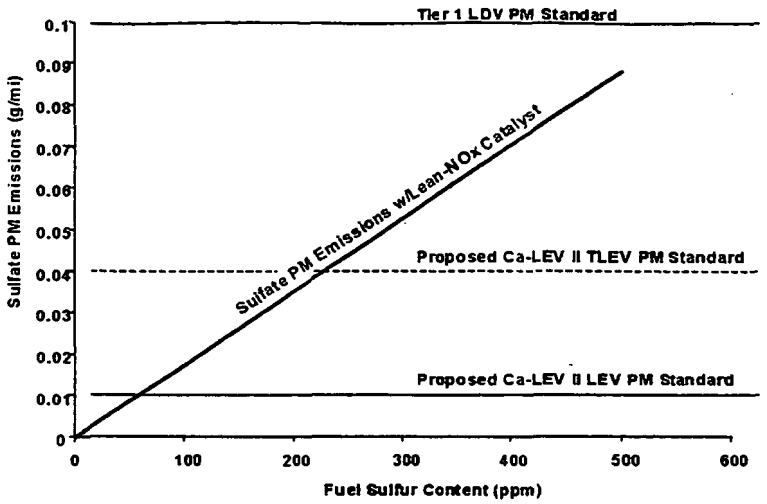


Figure 2-8. Estimated sulfate (primarily H_2SO_4) PM emissions from a LD truck equipped with a low-temperature Pt-zeolite lean-NO_x catalyst system (Wall, 1998).

compounds to be above their minimum detection limits. Because all of these devices incorporate oxidation catalyst functions to some extent, some of the comments related to oxidation catalysts also apply here. One major difference is that some of the aftertreatment devices rely on (lean-NO_x catalyst, NO_x-storage catalyst), or are sometimes assisted by (CRT), the introduction of additional fuel hydrocarbons, either as a reductant or to maintain a high exhaust temperature. The possible species formed from the oxidation or partial oxidation of fuel hydrocarbons have not been determined.

2.2.4. History of Dieselization

2.2.4.1. *Dieselization of the On-Road Fleet*

Understanding the prevalence of diesel engine penetration into the motor vehicle market is an important aspect of estimating the potential health effects of diesel emissions, past and present. Two classification systems based on rated gross vehicle weight are in use for trucks. These are listed below (Table 2-3).

Table 2-3. Vehicle classification and weights for on-road trucks

Class	Weight (lb)
3	10,001-14,000
4	14,001-16,000
5	16,001-19,500
6	19,501-26,000
7	26,001-33,000
8A[a]	33,001-60,000
8B[a]	>60,000
Medium duty (MD)	10,001-19,500 (same as Classes 3-5)
Light-heavy duty (LHD)	19,501-26,000 (same as Class 6)
Heavy-heavy duty (HHD)	>26,001 (same as Class 7-8)

[a]Class 8A and Class 8B are often considered together.

New diesel vehicle sales data for weight classes 5-8 are shown in Figure 2-9 for the years 1957-1998. The number of Class 7 and 8 diesel trucks sold has increased steadily with time while the number of smaller Class 5 and 6 trucks sold peaked in the 1960s and early 1970s and has since decreased. Retail and factory sales data show an increase in the percentage of diesel engines used in trucks sold in Classes 5-8. Using data from factory and retail sales, the percentage of diesel trucks sold by class is shown for the years 1957-1998 in Figure 2-10. The increase in the use of diesel relative to other fuels first occurred for Class 8 trucks. By 1983 more than 97% of the Class 8 trucks sold had diesel engines, according to Navistar and Motor Truck Facts (Bunn, 1999; AAMA Motor Vehicle Facts & Figures, 1983). Before 1980, about 60% of the Class 7 trucks were diesel but very few of the Class 5 and 6 trucks were diesel (≤ 16% combined). Use of diesel engines in these weight classes increased substantially in the 1980s, with roughly 80% of Class 6 and 67% of Class 7 trucks sold in 1997 being diesel.

Additional insight into dieselization of the on-road fleet can be gained from the 1992 U.S. Census of Transportation (1995). A summary of results is presented in Table 2-4. These data indicate that in 1992 the Class 7 plus Class 8 fleet was 88% diesel. The data presented in Figure 2-10 for the combined fleet in 1992 are in agreement with this value. Dieselization for Class 6 in 1992 was only 37% and for Classes 3-5 only 26%.

The 1992 Census of Transportation also provides information on the model year distribution for vehicles of various weight classes (Figure 2-11). A few 1993 model year

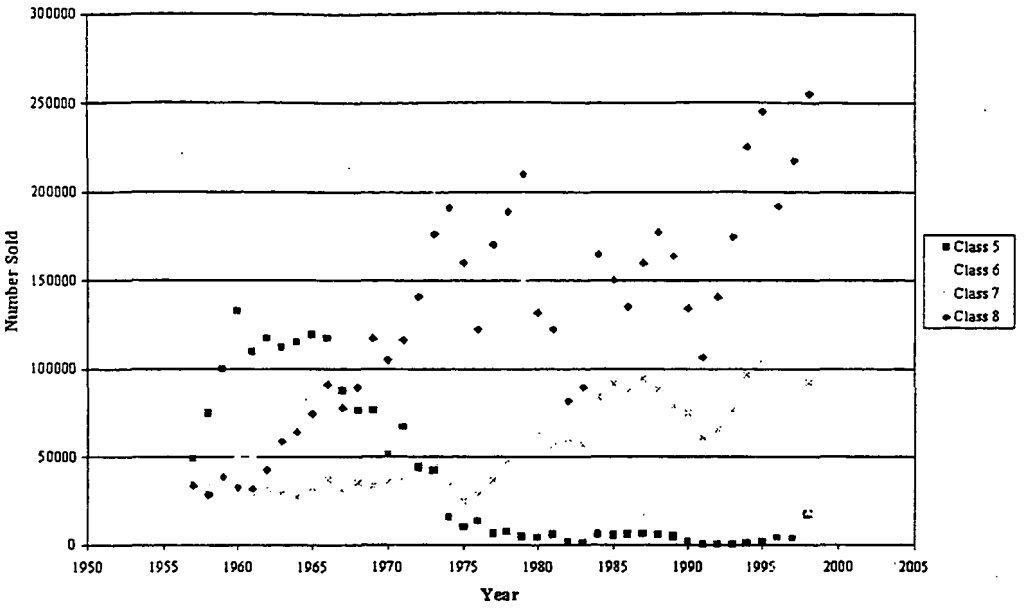


Figure 2-9a. Number of HD diesel trucks sold in years 1957-1998 based on industry sales data.
Source: Bunn (1999).

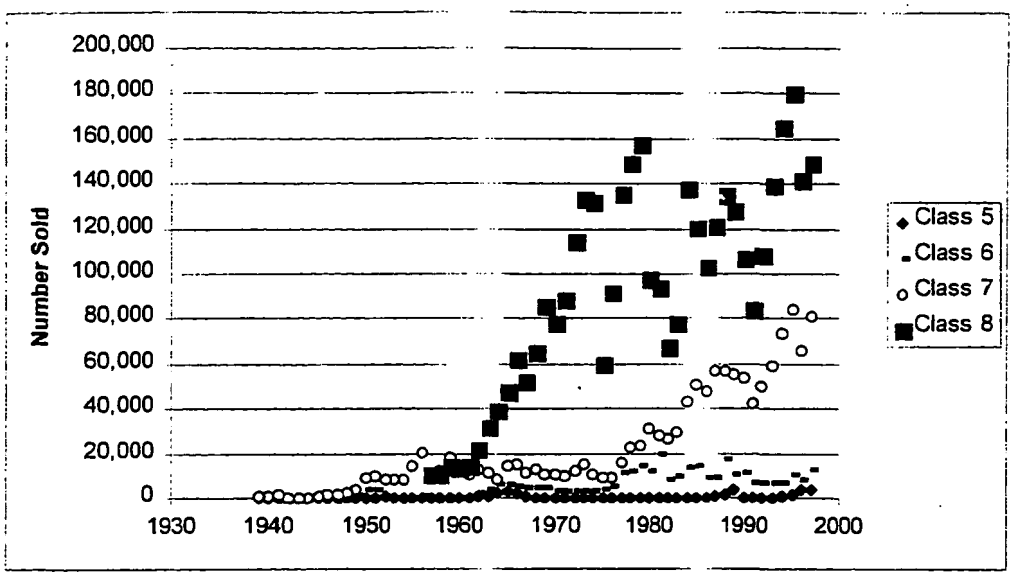


Figure 2-9b. Diesel truck sales (domestic) for the years 1939-1997.
Source: AMA/AAMA Motor Truck Facts.

Table 2-4. Truck fleet results for 1992 from Census of Transportation (1995), results in thousands

Truck class	1992 trucks	1992 diesel	% Diesels
Class 3, 4. and 5 (Medium duty)	1,259.0	326.3	25.9
Class 6 (Light heavy-duty)	732.0	269.7	36.8
Class 7 and 8 (Heavy heavy-duty)	1,966.2	1725.3	87.8

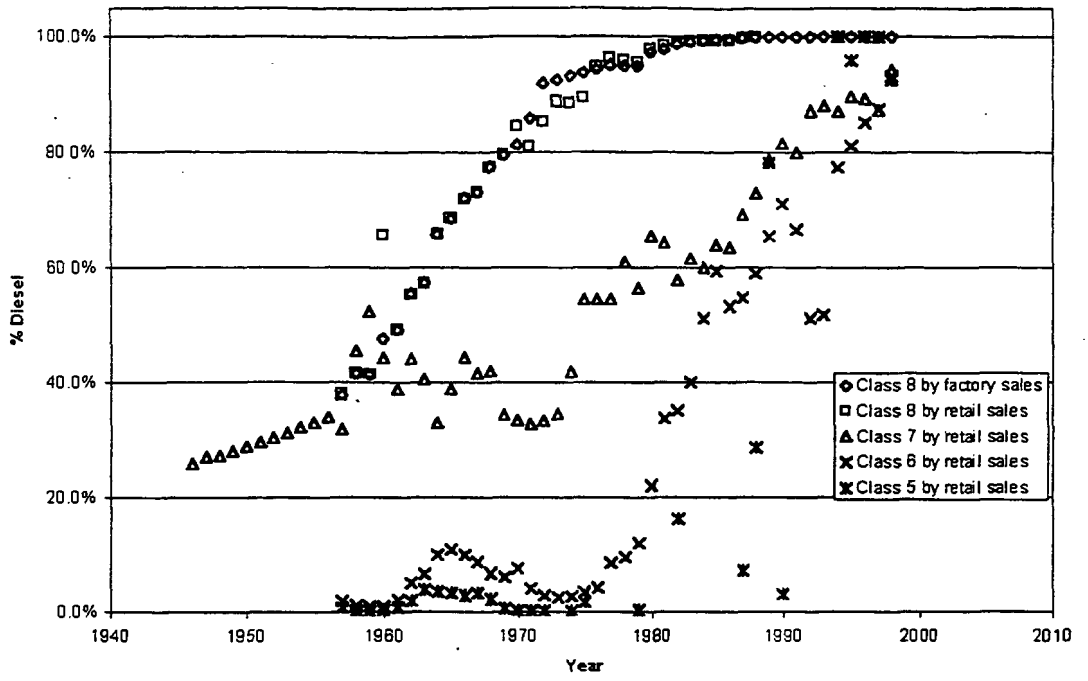


Figure 2-10a. Diesel truck sales as a percentage of total truck sales for the years 1957-1998. Source: Bunn (1999).

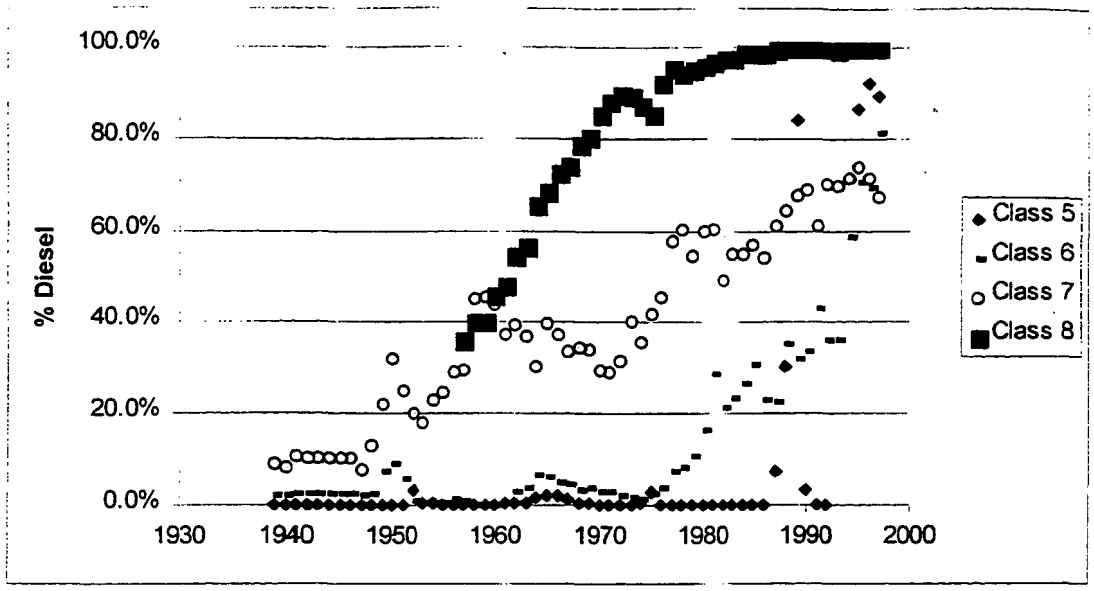


Figure 2-10b. Diesel truck sales as a percentage of total truck sales for the years 1939-1997.
Source: AMA/AAMA Motor Truck Facts.

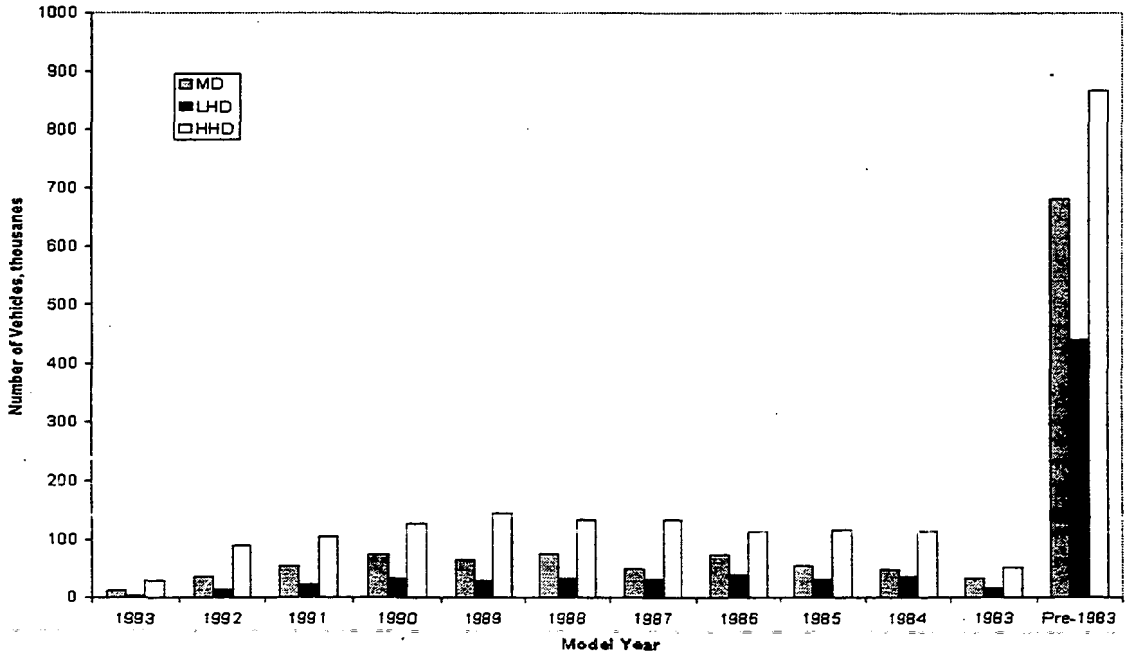


Figure 2-11. Model year distribution of in-use truck fleet in 1992.
Source: Census of Transportation, 1995.

vehicles were in use in 1992. For all three vehicle classes in 1992 there were a large number of vehicles more than 10 years old: 54% for medium duty, 60% for light HD, and 43% for heavy HD. For heavy HD trucks (Classes 7 and 8) there are roughly 100,000 vehicles in each model year from 1983 to 1993. Assuming this is also true before 1983, a large number of trucks (400,000) must be more than 14 years old. This suggests a truck life of around 18 years. The EPA MOBILE5 model assumes a vehicle life of 20 years and MOBILE6 assumes a vehicle life of 25 years, with aging vehicles having fewer vehicle miles traveled on an annual basis (U.S. EPA, 1999a). Motor Truck Facts and later American Automobile Association "Facts and Figures" (AMA, 1927-1975), indicate that 53% of trucks from model years 1947-1956 were still on the road after 14 years and 55% of trucks from model years 1960-1969 were still on the road after 14 years. The proportion of trucks in use after 14 years is 63% for model years 1974-1983, suggesting that the lifespan of trucks built in later years is longer.

In the years since 1950 to 1990 and beyond, vehicle miles traveled by all types of vehicles have increased significantly. For example, Department of Transportation Federal Highway Administration statistics show that passenger car vehicle miles traveled increased from about 400 billion in 1951 to 1,400 billion in 1995 and 1,500 billion in 1997, an increase of about 360% and 380% for these years compared to 1951. Meanwhile, vehicle miles traveled by combination trucks increased from about 20 billion in 1951 to 94,000 billion in 1990 and 124,500 billion in 1997, a somewhat larger increase of 470% and 620% for these years compared to 1951. These data highlight the fact that combination truck usage has increased more than passenger car usage from the early 1950s to the 1990 and 1997 time frame. The Department of Transportation statistics are also available for other vehicle categories such as lighter trucks.

The EPA MOBILE5 and PART5 models calculate that about 2.6% of total vehicle miles traveled in the 1950 time frame came from diesels with a gross vehicle weight over 33,000 pounds (Classes 7 and 8). In 1990, about 3.3% of total vehicle miles traveled came from diesel trucks in these weight categories. In the 1950-1990 time frames and beyond, diesel trucks are responsible for an increasing fraction of the vehicle miles traveled.

2.2.4.2. *Dieselization of Railroad Locomotive Engines*

Early in the 20th century the political and economic pressure on the railroads to replace steam locomotives was substantial. Railroads were losing business to other forms of transport. The diesel-electric locomotive provided 90% in-service time compared to only 50% for steam locomotives, and had three times the thermal efficiency (Klein, 1991; Kirkland, 1983). Additionally, several cities had passed laws barring steam locomotives within the city limits because the large quantities of smoke obscured visibility, creating a safety hazard. The first

prototype diesel locomotive was completed in 1917. By 1924 General Electric was producing a standard line of switching locomotives on a production basis. Electro-Motive Corporation was founded the same year to produce diesel locomotives in competition with GE. This company was purchased in 1929 by General Motors and became the Electro-Motive Division. After this acquisition, GM began to develop the 2-stroke engine for this application. Up to this time, all locomotive diesel engines were 4-stroke. Two-strokes offered a much higher power-to-weight ratio and GM's strategy was to get a large increase in power by moving to the 2-stroke cycle. The first true high-speed, 2-stroke diesel-electric locomotives were produced by GM in 1935. However, because of the economic climate of the Great Depression few of these were sold until after the Second World War. At the end of the war most locomotives were still steam-driven but were more than 15 years old, and the railroads were ready to replace the entire locomotive fleet. Few if any steam locomotives were sold after 1945 as the entire fleet was converted to diesel (Coifman, 1994).

The locomotive fleet has included significant percentages of both 2- and 4-stroke engines. The 4-stroke diesel engines were naturally aspirated in the 1940s and 1950s. It is unlikely that any of the 2-stroke engines used in locomotive applications were strictly naturally aspirated. Nearly all 2-stroke diesel locomotive engines are uniflow scavenged, with a positive-displacement blower for scavenging assistance. In 1975, it was estimated that 75% of the locomotives in service were 2-stroke, of which about one-half used one or more turbochargers in addition to the existing positive-displacement blower for additional intake boost pressure.

Almost all of the 4-stroke locomotive engines were naturally aspirated in 1975 (Hare and Springer, 1972). Electronic fuel injection for locomotive engines was first offered in the 1994 model year (U.S. EPA, 1998b). All locomotive engines manufactured in recent years are turbocharged, aftercooled or intercooled 4-stroke engines. In part, this is because of the somewhat greater durability of 4-strokes, although impending emissions regulations may have also been a factor in this shift. The typical lifespan of a locomotive has been estimated to be more than 40 years (U.S. EPA, 1998b). Many of the smaller railroads are still using engines built in the 1940s, although the engines may have been rebuilt several times since their original manufacture.

2.2.4.3. *Historical Trends in Diesel Fuel Use and Impact of Fuel Properties on Emissions*

Use of diesel fuel has increased steadily in the second half of this century. According to statistics from the Federal Highway Administration (1995; 1997a), in 1949 diesel fuel was approximately 1% of the total motor fuel used, and in 1995 it was about 18%. Over the same

time diesel fuel consumption increased from about 400 million gallons to 26 billion gallons per year in the United States, an increase by a factor of more than 75 (Figures 2-12 and 2-13).

The chemistry and properties of diesel fuel have a direct effect on engine emissions. Researchers have studied the effect of sulfur content, total aromatic content, polyaromatic content, fuel density, T90/T95, oxygenate content, and cetane. Lee et al. (1998) have comprehensively reviewed literature studies of the effect of these fuel properties on regulated emissions. Their conclusions were based on fleet and multiple engine tests conducted over both transient and steady-state cycles, and were limited to studies in which the effects of the various fuel characteristics could be decoupled from each other. Sulfur content, cetane number, density, total aromatics and polyaromatics content, as well as boiling point distribution can have an impact on emissions. It was concluded that the effect of most fuel changes on modern engines is less than the effect on older, higher emitting engines.

Most important for emissions, the chemical makeup of diesel fuel has changed over time, in part because of new regulations. EPA currently regulates diesel fuel and requires sulfur content to be less than 500 ppm for on-road applications, and that cetane index (a surrogate for actual measurements of cetane number) be greater than or equal to 40, or the maximum aromatic content to be 35% or less (CFR 40:80.29). California has placed additional restrictions on the cetane number and aromatic content of diesel fuel (California Code of Regulations, Title 13).

Prior to 1993, diesel fuel sulfur levels were not federally regulated in the United States. Only recommended industry practices were in place (e.g., the ASTM D 975 specified 0.5% fuel sulfur limit). During the years 1960 to 1986, fuel sulfur content showed no chronological increasing or decreasing trends and ranged from 0.23-0.28wt%, while the average cetane number of U.S. diesel fuel declined steadily from 50.0 to 45.1, or about 0.2 per year (NIPER, 1986). Based on a linear regression analysis, the average cetane number was 52.2 in 1949 and 46.8 in 1976. This declining trend in cetane number was likely accompanied by an increase in aromatic content and density (Lee et al., 1998). The reason for the decline was that as diesel demand grew, straight-run diesel became a smaller part of the pool and light-cycle oil from catalytic cracking became important. Light-cycle oil is high in aromatics. One study measuring the impact of changes in cetane number and aromatic content found that increasing the aromatic content from 20% to 40%, with an accompanying decrease in the cetane number from 53 to 44 resulted in a 4% increase in NO_x and a 7% increase in PM (McCarthy et al., 1992). These values can be considered reasonable upper bounds for the small effect changes in fuel quality likely had on NO_x and PM emissions during the years 1949-1975.

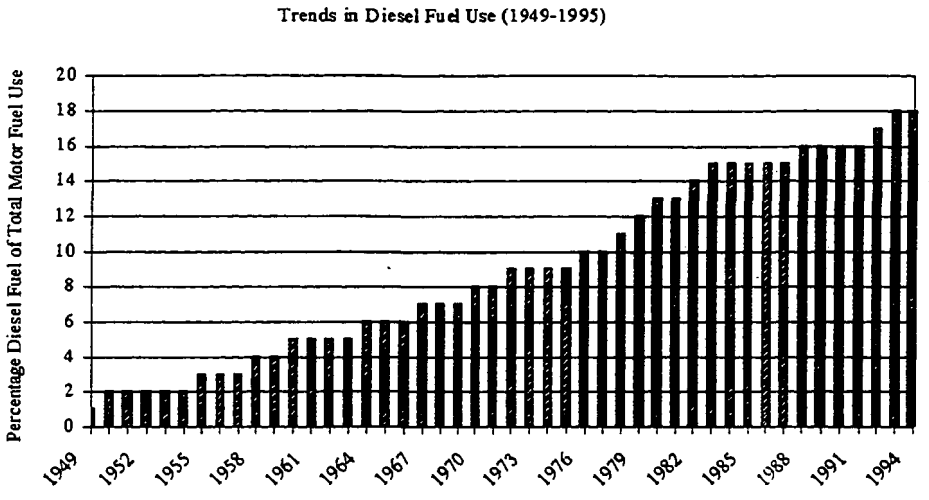


Figure 2-12. Diesel fuel use since 1949.
Source: Federal Highway Administration, 1995.

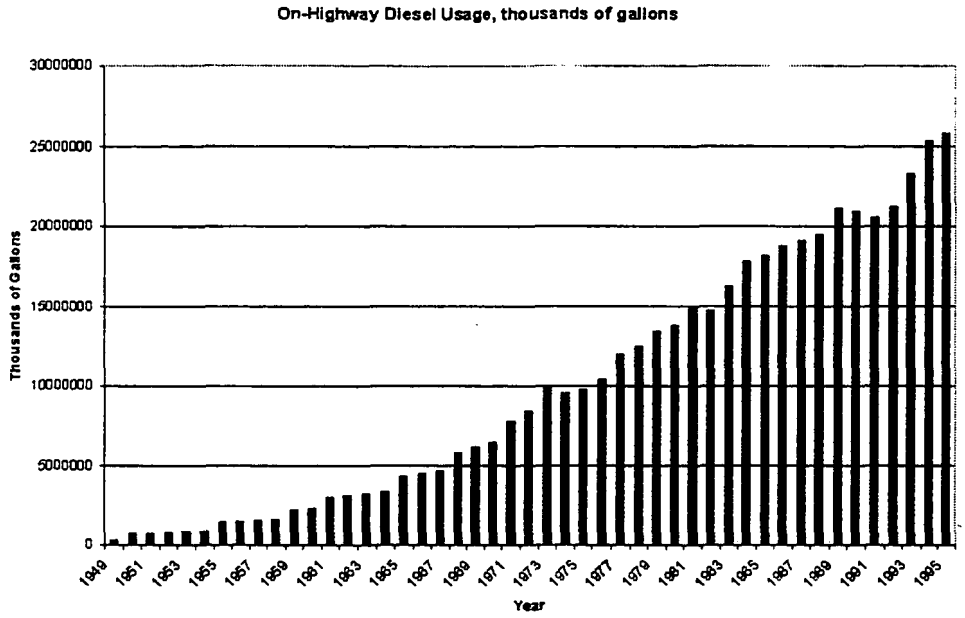


Figure 2-13. On-highway diesel fuel consumption since 1949, values in thousands of gallons.
Source: Federal Highway Administration, 1995.

In wintertime, on-road No. 2 diesel may contain some percentage (~15% or more) of No. 1 diesel to improve cold flow properties. Blending of No. 1 may also lower the aromatic content, resulting in improved emissions performance. Thus, there may also be some small but perceptible seasonal changes in emissions from diesel engines.

A maximum allowable fuel sulfur content in the United States for on-road diesel fuel was established at 0.05 mass % in 1993 in advance of a new 0.10 g/bhp-hr PM standard for HD on-highway trucks. This reduced total PM emissions through reduction of sulfate PM (primarily present as sulfuric acid). Approximately 1% to 4 % of fuel sulfur is oxidized to SO_3, which forms sulfuric acid in the presence of water vapor in the exhaust (Wall et al., 1987; Khatri et al., 1978; Baranescu, 1988). Considerably higher sulfuric acid PM emissions are possible with diesel exhaust aftertreatment systems containing precious metals (oxidation catalysts, lean NO_x catalysts, catalyzed PM traps). At temperatures over 350 to 500 °C (depending on device), SO_2 in the exhaust can be oxidized to SO_3 and increase sulfuric acid PM emissions (McClure et al., 1992; McDonald et al., 1995; Wall, 1998). Sulfur content remains at unregulated levels for off-highway diesel fuels. Nationally, on-road fuels averaged 0.032% sulfur in 1994 while off-highway fuels averaged 0.322% (Dickson and Sturm, 1994).

2.2.5. Chronological Assessment of Emission Factors

2.2.5.1. *On-Road Vehicles*

Historically, measured emissions from HD diesel vehicles have been widely variable. However, certain chronological trends can be identified, driven primarily by tightening regulatory standards since the mid-1970s. Prior to that time, changes in average fuel composition and engine technologies were implemented for reasons other than emissions control. Although there is a reasonable amount of data upon which to base an emission factor for late 1970s and later engines, there are virtually no transient test data available to EPA on engines earlier than the mid-1970s. Nevertheless, there are some factors that help lead to conclusions about the emissions of these engines. Diesel truck engine technology changed little in this time frame, and over this whole period used roughly the same means of tuning the engine's air-fuel ratio; that is, tuning was done to not permit air-fuel ratios richer than the "smoke limit" of about 22:1. This tuning, in essence, formed an "upper limit" on particulate emissions and was done before EPA smoke standards (for customer satisfaction reasons). There is only qualitative correlation between smoke and particulate emissions over the transient driving cycle, but there is semiquantitative correlation between smoke and particulates over steady-state operating modes (McGuckin and Rykowski, 1981). The fact that engines, turbocharged or not, were tuned to avoid smoky operation makes it reasonable to assume that they had emissions roughly at the

mid-1970s level. Other than the increased use of turbochargers, HD diesel engine technology was reasonably stable. Thus, it is reasonable to conclude that the emission factors developed above for mid-1970s (and later) engines adequately represent the engines in use in the 1950-1970 time frame.

There have been numerous studies of emissions from in-use on-road HD (greater than 8,500 lbs GVWR) diesel vehicles. Emissions of regulated pollutants from these studies have been reviewed (Yanowitz et al., 1999b) and the review findings, which encompass vehicles from model years 1976 to 1998, are summarized below.

Figures 2-14 through 2-16 below show chassis dynamometer data from more than 200 different vehicles, reported in 20 different published studies (approximately half of which are from transit buses) (Yanowitz et al., 1999a; Warner-Selph and Dietzmann, 1994; Dietzmann et al., 1980; Graboski et al, 1998a; McCormick et al., 1999; Clark et al., 1997; Bata et al., 1992; Brown and Rideout, 1996, Brown et al., 1997; Clark et al., 1995; Dunlap et al., 1993; Ferguson et al., 1992; Gautam et al., 1992; Katragadda et al., 1993; Rideout et al., 1994; Wang et al., 1993; Wang et al., 1994; Williams et al., 1989; Whitfield and Harris, 1998), as well as a large amount of additional data collected by West Virginia University and available on the World Wide Web at www.afdc.nrel.gov. The results from vehicles tested more than once using the same test cycle, and without any additional mileage accumulated between tests, are averaged and reported as one data point. Emissions results from vehicles tested under different test cycles or at different points in the engine's life cycle have been reported as separate data points. Note that all NO_x mass emissions data are reported as equivalent NO_2.

Figures 2-14 through 2-16 show emissions trends for NO_x, PM, and HC in g/mile. A least-squares linear regression is plotted on each graph and yields the following equations for predicting emissions trends (applicable to the years 1976-1998):

$$\text{Log } NO_x \text{ (g/mile)} = (\text{Model year} * -0.008) + 16.519 \quad (2\text{-}1)$$

$$\text{Log PM (g/mile)} = (\text{Model year} * -0.044) + 88.183 \quad (2\text{-}2)$$

$$\text{Log HC (g/mile)} = (\text{Model year} * -0.055) + 109.390 \quad (2\text{-}3)$$

As shown in Figures 2-14, 2-15, and 2-16, which include 95% confidence limits and regression lines, changes in NO_x emissions have been relatively small, with an emission rate averaging about 26 g/mile. PM, CO, and THC emissions, though widely variable within any model year, have shown a pronounced declining trend. PM emissions from chassis dynamometer tests decreased from an average 3.0 g/mi in 1977 to 0.47 g/mi in 1997, suggesting a decrease in PM emissions of a factor of 6. Although it is clear that emissions of CO, HC, and

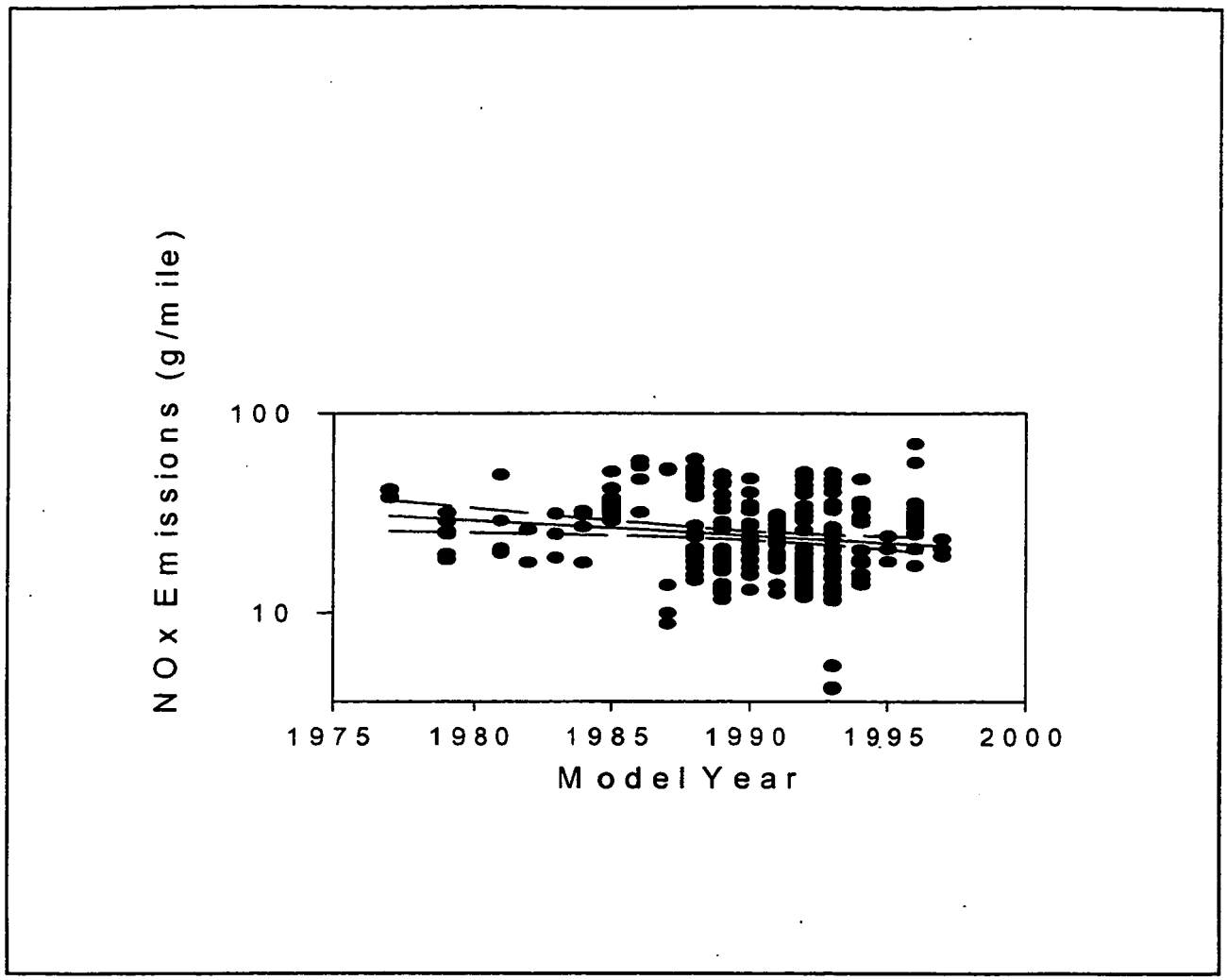


Figure 2-14. Model year trends in NO_x emissions (g/mile).

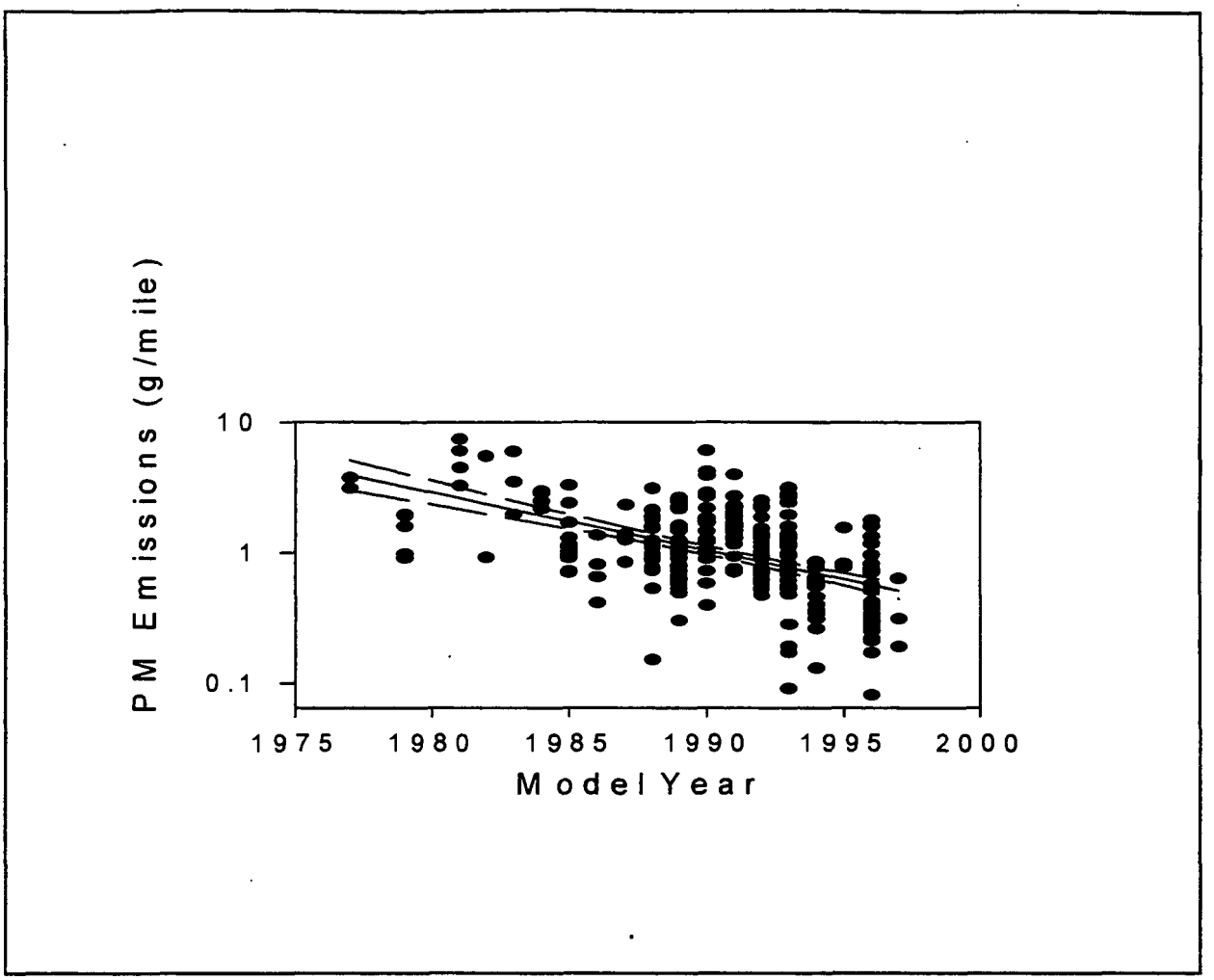


Figure 2-15. Model year trends in PM emissions (g/mile).

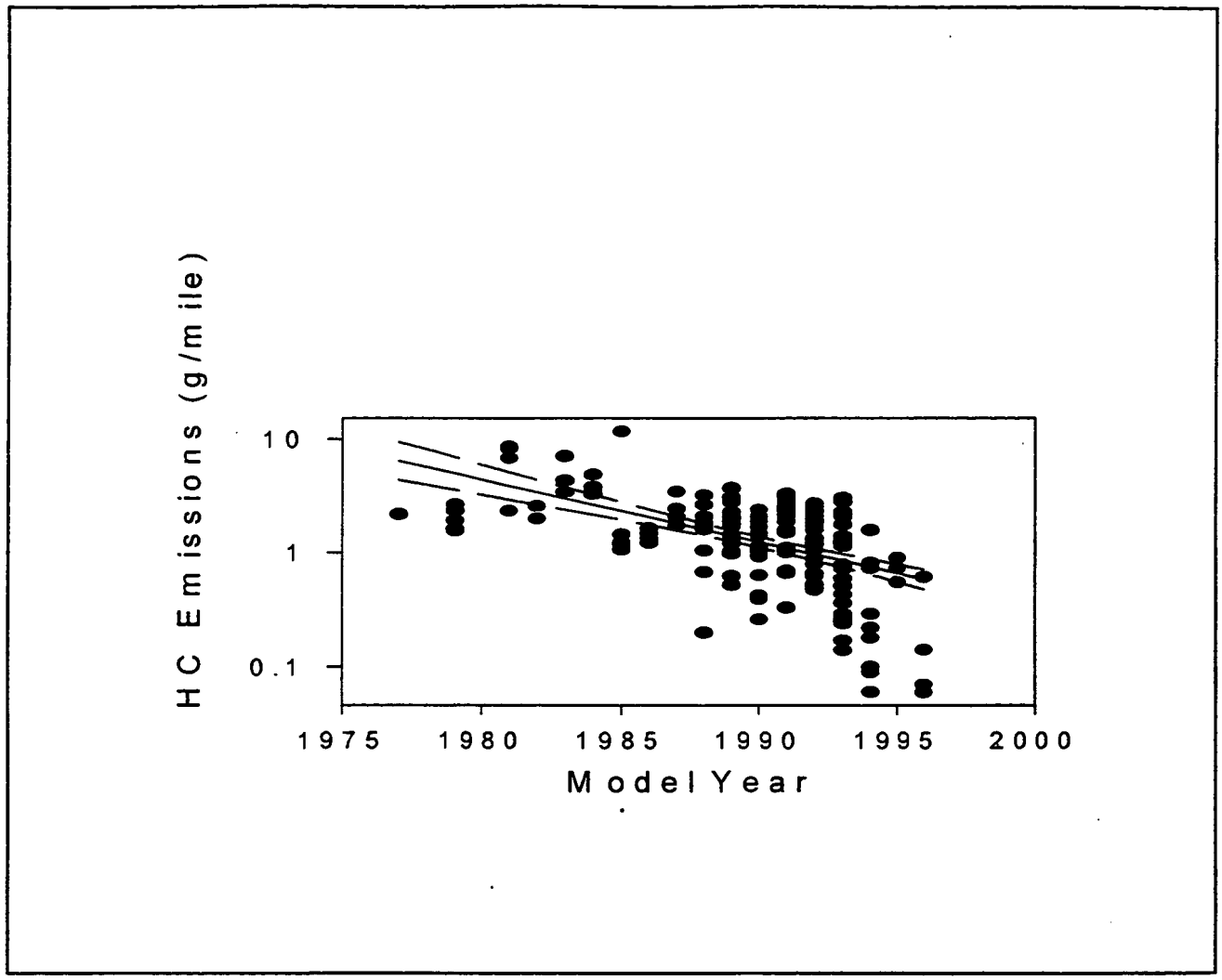


Figure 2-16. Model year trends in HC emissions (g/mile).

PM have declined significantly since the early 1970s, emissions of NO_x have remained approximately constant.

Other approaches for measuring emissions from in-use, on-road diesel vehicles include remote sensing and tunnel tests. The literature reports of those studies are summarized in Tables 2-5 and 2-6. Gram-per-mile emission factors vary substantially for the various tunnels, with NO_x ranging from 9 to 24 g/mile, PM ranging from 0.6 to 1.8 g/mile, CO ranging from 6 to 14 g/mile, and THC ranging from 0.16 to 2.55 g/mile. Remote sensing produces results in terms of pollutant emissions per unit of fuel, not on a per-mile basis. On a g/gallon of fuel consumed basis, agreement between the studies for NO_x emissions is reasonably good, suggesting an average level for the fleet of about 130 g/gal for both tunnel tests and remote sensing, comparable to the average emissions factor generated from chassis dynamometer studies. Generally, chassis dynamometer tests and engine dynamometer test results are corrected for ambient humidity in accordance with the Federal Test Procedure (CFR 40, Subpart N). Tunnel tests and remote sensing tests have typically not included corrections for humidity. Appropriate humidity corrections for NO_x and PM can be greater than 20% and 10%, respectively (or a total difference of more than 45% and 20% between low- and high-humidity areas), under normally occurring climatic conditions. Additionally, the remote sensing literature has not addressed how to determine the correct value for the NO/NO_x ratio, and there is reason to believe that this value may differ systematically from site to site although, again, most of the NO_x is NO.

There are no reported instances of HD diesel PM measurement by remote sensing, but there were several tunnel tests that measured PM. In addition to the humidity correction discussed above, several factors must be taken into account when comparing PM measurements from tunnel tests to the chassis dynamometer measurements (Yanowitz et al., 1999b): (1) Chassis dynamometer testing measures only tailpipe emissions; tunnel tests include emissions from other sources (tire wear, etc). (2) Tunnel tests typically measure emissions under steady-speed freeway conditions, whereas most chassis dynamometer tests are measured on cycles that are more representative of stop-and-go urban driving conditions. This latter limitation also applies to remote sensing readings, which measure instantaneous emissions versus emissions over a representative driving cycle. PM is emitted from HD vehicles at the greatest rate during accelerations.

Because THC emissions for diesel vehicles are very low in comparison to gasoline vehicles, tunnel test results for THC have a high degree of uncertainty. A regression analysis to determine the contribution of the limited number of HD vehicles to THC emissions is somewhat unstable, i.e., small errors in the total measurements can change estimates. Similarly, CO emissions are comparable to automobile emissions on a per-vehicle-mile basis, but since there

Table 2-5. Emissions results from tunnel tests (adapted from Yanowitz et al., 1999b)

Test	Tunnel Location	Fuel efficiency (mi/gal)	NO_x[a] (g/mi)	NMHC (g/mi)	CO (g/mi)	PM (g/mi)	CO2 (g/mi)	NO_x[a] (g/gal)	NMHC (g/gal)	CO (g/gal)	PM (g/gal)
Pierson and Brachaczeck, 1983	Allegheny, 1970-74	5.42[b]				.90-1.80					4.9-9.8
	Allegheny, 1975					1.75 +/-.19					9.49+/-1.03
	Allegheny, 1976					1.5 +/-.10					8.1 +/- .54
	Allegheny, 1976					1.4 +/-.07					7.6 +/-.4
	Tuscarora, 1976					1.3 +/-.19					7.0 +/-1.0
	Tuscarora, 1976					1.39 +/- 26					7.5 +/-1.40
	Allegheny, 1977					1.3 +/- .08					7.0 +/-.43
	Allegheny , 1979					1.2 +/- .03					6.5 +/-.16
	Allegheny, 1979					1.4 +/- .04					7.6 +/-.19
Rogak et al., 1998	Cassiar Tunnel, 1995, Vancouver	8.03[b]	19.50 +/- 4.22	-0.16 +/- 0.88	6.79 +/- 11.78		1280 +/- 40	157 +/- 34	-1 +/- 7	55 +/- 95	
Miguel et al., 1998	Caldecott Tunnel, 1996, San Francisco	5.42[c]	23.82 +/- 4.17			1.67 +/- 0.24[d]		129 +/- 23			9.0 +/- 1.3[d]
Weingartner et al., 1997	Gubrist Tunnel, 1993, Zurich	5.60[e]				0.62 +/- 0.02[f]					3.5 +/- 0.1[f]
Pierson et a., 1996	Fort McHenry Tunnel, downhill, 1992, Baltimore	11.46[b]	9.66 +/- 0.32	0.92 +/- 0.21	6.8 +/- 1.5		897 +/- 48	111 +/- 4	11 +/- 2	78 +/- 17	
Pierson et a ., 1996	Fort McHenry Tunnel, uphill, 1992, Baltimore	5.42[b]	22.50 +/- 1.00	2.55 +/- 1.05	14.3 +/- 5.5		1897 +/- 168	122 +/- 5	14 +/- 6	78 +/- 30	
Pierson et a ., 1996	Tuscarora Tunnel 1992, Pennsylvania	6.44[b]	19.46 +/- 0.85	0.68 +/- 0.20	6.03 +/- 1.61		1596 +/- 78	125 +/- 5	4 +/- 1	39 +/- 10	
Kirchstetter et al., 1999	Caldecott Tunnel, 1997, San Francisco	5.42[c]	23.82 +/- 2.98			1.43 +/- 0.12[g]		129 +/- 16			7.7 +/- 0.6[g]

[a] NO_x reported as NO_2.

[b] Calculated from observed CO_2 emissions assuming fuel density 7.1 lb/gal and C is 87% of diesel fuel by weight.

[c] Since CO_2 emissions not available, fuel efficiency assumed to be the same as in slightly uphill tunnel (Fort McHenry).

[d] Reported as black carbon, assumed that 50% of total PM emissions are BC.

[e] Slope of tunnel unknown, so used average fuel efficiency for the United States.

[f] PM_3.

[g] $PM_{2.5}$.

[h] Uncertainty reported as +/-1.0 standard deviation, except where literature report did not specify standard deviation; in those cases uncertainty listed as reported.

Table 2-6. Remote sensing results for hd vehicles (Yanowitz et al., 1999b)

	Reference	Year study conducted	Emissions (g/gal)
NO_x (reported as NO_2)	Jimenez et al., 1998	1997	150 [a,b,c]
	Cohen et al., 1997	1997	108 [a,b,c]
	Countess et al., 1999	1998	187 [a,b,c]
CO	Bishop et al., 1996	1992	59 [b]
	Cohen et al., 1997	1997	54 [b]
	Countess et al., 1999	1998	85 [b]
THC	Bishop et al., 1996	1992	0.002 HC/CO_2 mole ratio [d]
	Cohen et al., 1997	1997	0.00073 HC/CO_2 mole ratio [d]

[a]Remote sensing measures NO. The reported value was corrected to a NO_x (as NO_2) value by assuming 90% (mole fraction) of NO_x is NO.
[b]Emissions in g/gal calculated by assuming that fuel density is 7.1 lb/gal and C is 87% by weight of fuel.
[c]No humidity correction factor is included.
[d]In order to calculate emissions in g/gal, an average molecular weight is needed.

are generally many more automobiles than HD diesels in tunnel tests, CO measurements may be associated with a high degree of uncertainty.

Given the variability between testing methods, and assuming the on-road fleet measured in tunnel and remote sensing tests is primarily vehicles 0 to 10 years old, there is reasonable comparability between chassis dynamometer results and average tunnel and remote sensing results for PM, CO, and HC. Neither tunnel testing nor remote sensing results show any pronounced chronological trends for these regulated pollutants, primarily because virtually all of the testing was done over a short period in the 1990s. The average model year for HD vehicles was reported in only one case (Kirchstetter et al., 1999) and was found to be 1988 for a tunnel test conducted in 1997. However, even in the case of tunnel testing, measurements of PM from the 1970s PM levels are not significantly different from what was measured in the 1990s.

The data on regulated emissions do allow a comparison of emissions from 2- and 4-stroke engines for PM (Figure 2-17). It is clear that there is no significant difference in PM mass emissions for 2- and 4-stroke engines over the time period covered. This is true even in 1993. In model year 1994 there were no on-road 2-stroke engines. Similarly, no significant difference was observed for emissions of HC, CO, or NO_x.

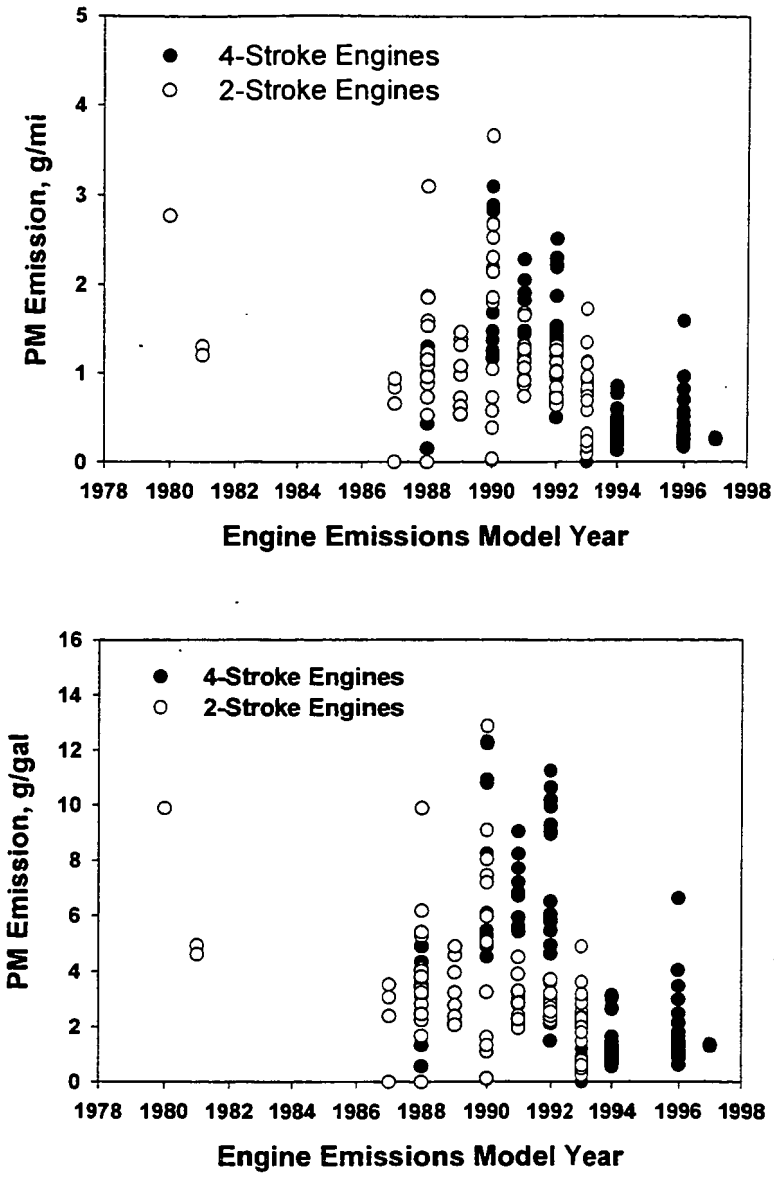


Figure 2-17. Comparison of 2-stroke and 4-stroke engines PM emissions on a g/mi and g/gal basis (low altitude data only).
Source: Yanowitz et al., 1999b.

2.2.5.2. *Locomotives*

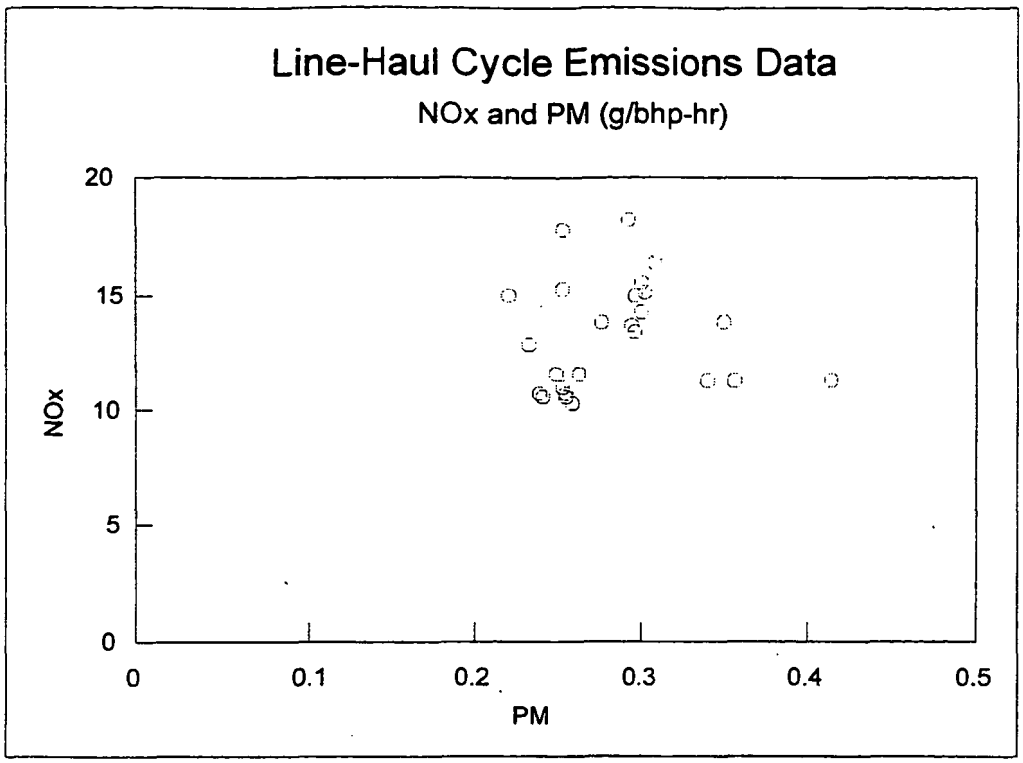
Locomotive engines generally range from 1000 horsepower up to 6000 horsepower. Similar to the much smaller truck diesel engines, the primary pollutants of concern are NO_x, PM, CO, and HC. Unlike truck engines, most locomotive engines are not mechanically coupled to the drive wheels. Because of this decoupling, locomotive engines operate in specific steady-state modes rather than the continuous transient operation normal for trucks. Because the locomotive engines operate only at certain speeds and torques, the measurement of emissions is considerably more straightforward for locomotive engines than for truck engines. Emissions measurements made during the relatively brief transition periods from one throttle position to another indicate that transient effects are very short and thus could be neglected for the purposes of overall emissions estimates.

Emissions measurements are made at the various possible operating modes with the engine in the locomotive, and then weighting factors for typical time of operation at each throttle position are applied to estimate total emissions under one or more reasonable operating scenarios. In the studies included in this analysis, two scenarios were considered: line-haul (movement between cities or other widely separated points) and switching (the process of assembling and disassembling trains in a switchyard).

The Southwest Research Institute made emissions measurements for three different engines in locomotives in 1972 (Hare and Springer, 1972) and five more engines in locomotives using both low- and high-sulfur fuel in 1995 (Fritz, 1995). Two engine manufacturers (the Electromotive Division of General Motors, or EMD, and the General Electric Transportation Systems, or GETS) tested eight different engine models and reported the results to EPA (U.S. EPA, 1998b). There are also additional data. All available data on locomotives are summarized in the regulatory impact assessment and shown in Figure 2-18.

2.2.6. Physical and Chemical Composition of Particles

Diesel PM is defined by the measurement procedures summarized in Title 40 CFR, Part 86, subpart N. These procedures define PM emissions as the mass of material collected on a filter at a temperature of 52 °C or less after dilution of the exhaust. As the exhaust is diluted and cooled, nucleation, condensation, and adsorption transform volatile material to solid and liquid PM. Diesel exhaust particles are aggregates of primary spherical particles consisting of solid carbonaceous material and ash, and which contain adsorbed organic and sulfur compounds (sulfate) combined with other condensed material. The organic material includes unburned fuel, lube oil, and partial combustion and pyrolysis products. This is frequently quantified as the soluble organic fraction, or SOF. The SOF can range from less than 10% to more than 90% by



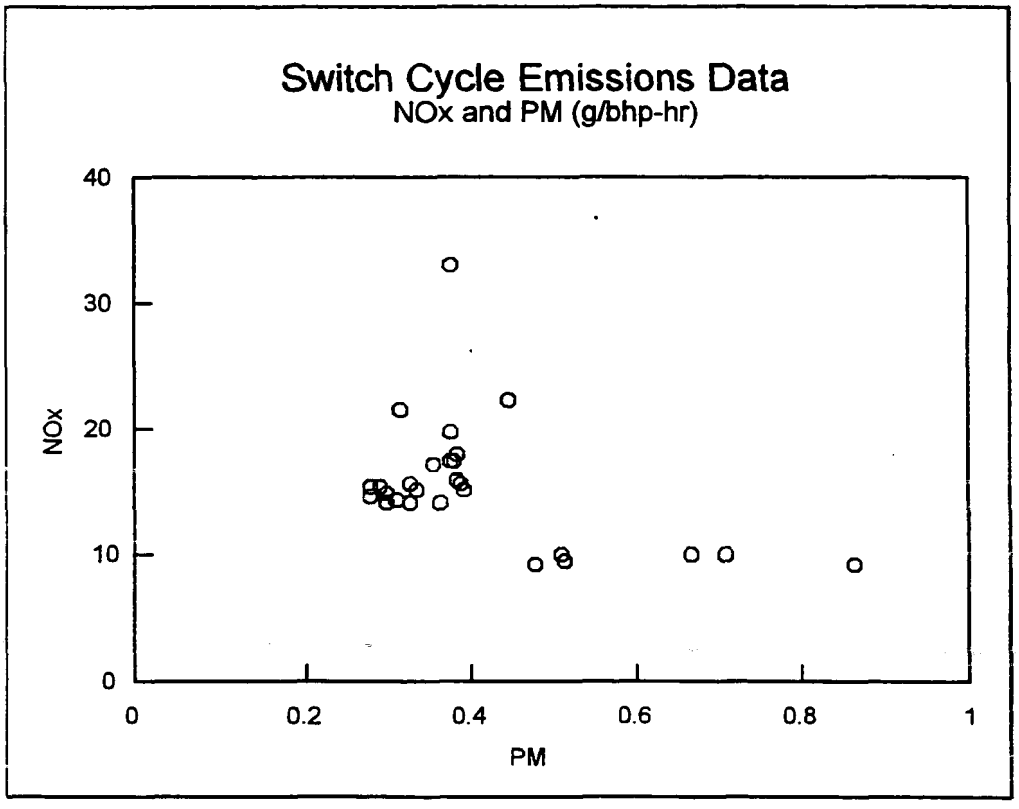


Figure 2-18. Line-haul and switch emissions data.
Source: U.S. EPA, 1998a.

mass, with the highest values occurring at light engine load where exhaust temperature is low (Kittelson, 1998). The SOF fraction can also vary with engine design, with high lube oil emitting older engines producing higher SOF. Sulfate depends on fuel sulfur content primarily.

Carbonaceous diesel particulate matter has a high specific surface area (30-50 m^2/g) (Frey and Corn, 1967). Because of this high surface area diesel particles are able to adsorb large quantities of organic materials. After removal of the organic material by extraction, the surface area increases to as high as 90 m^2/g (Pierson and Brachaczek, 1976). A variety of solvents have been used to extract the SOF (Levson, 1988). Soxhlet extraction with a binary solvent consisting of an aromatic and an alcohol appears to give the best recovery of PAHs, although dichloromethane is also used. Some studies have then used liquid chromatography to separate the extract into various fractions on the basis of chemical composition and polarity.

The distribution of emissions between the gas and particle phases in diesel exhaust (gas/particle partitioning) is determined by the vapor pressure of the individual species, the type and amount of particulate matter present (surface area available for adsorption), and the temperature (Zielinska et al., 1998). Two-ring and smaller compounds exist primarily in the gas phase while five-ring and larger compounds are completely adsorbed on the particles. Three- and four-ring compounds are distributed between the two phases. Some studies use sampling trains designed to collect both gas-phase and particle-phase compounds, while others simply report the amount or emission of a given compound in the PM SOF. During the collection of particulate and organic compounds, filter adsorption, blow-off (loss from the filter), and chemical transformation of the semivolatile compounds have been reported to occur (Schauer et al., 1999; Cantrell et al., 1986; Feilberg et al., 1999; Cautreels and Cauwenberghe, 1978).

For diesel engine emissions, approximately 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} (Black and High, 1979; Pierson et al., 1983). Polycyclic aromatic hydrocarbons and alkyl-substituted PAHs account for the remainder of the nonpolar mass. The moderately polar fraction (~9% w/w of extract) consists mainly of oxygenated PAHs species, substituted benzaldehydes, and nitrated PAHs. The polar fraction (~32% w/w of extract) is composed mainly of n-alkanoic acids, carboxylic and dicarboxylic acids of PAHs, hydroxy-PAHs, hydroxynitro-PAHs, nitrated N-containing heterocyclic compounds, etc. (Schuetzle, 1983; Schuetzle et al., 1985).

Rogge et al. (1993) reported the composition of the extractable portion of fine particulate matter emitted from two HD diesel trucks (1987 model year). No HPLC separation step was employed and the extract (hexane followed by benzene/2-propanol) was analyzed by capillary gas chromatography/mass spectrometry (GC/MS) before and after derivatization. The unresolved organic mass, which comprises 90% of the elutable organic mass, consists mainly of

branched and cyclic hydrocarbons. From the mass fraction that is resolved as discrete peaks by GC/MS, ~42% was identified as specific organic compounds. Most of the identified resolved organic mass (~60%) consists of n-alkane, followed by n-alkanoic acids (~20%). PAHs account for ~3.5% and oxy-PAHs (ketones and quinones) for another ~3.3%. Taking into account the differences in the analytical procedures and the percentage of identified peaks, this distribution is roughly similar to those reported by Schuetzle (1983).

2.2.6.1. *SOF and Elemental Carbon Content of Particles*

Chassis dynamometer results indicate that SOF emissions have trended downward over the years as engine manufacturers have tried to reduce oil consumption. This is shown in Figure 2-19, where the trend can be seen as both reduction in SOF weight percent and in SOF g/mi emissions. The downward trend is driven primarily by the need to reduce oil consumption, and thereby reduce engine wear and maintenance costs, as well as the need to meet PM emission standards. There is no significant difference in SOF emissions from 2- and 4-stroke engines in later years, while 2-stroke engines in the 1970s tended to emit greater amounts of SOF compared with typical 4-stroke engines. The downward trend in SOF as a percentage indicates that the solid carbonaceous material as a percentage of PM has been increasing. Figure 2-20 shows the PM solids g/mile emissions (TPM-SOF) for several vehicles tested on chassis dynamometers. A decreasing trend in PM solids is also evident, consistent with the observed decline in total PM emissions. It is tempting to assume that this solid carbonaceous portion is approximately the same as elemental carbon (EC, a quantity not commonly measured in HD studies). This assumption is validated, to a good approximation, by a study in which both SOF and EC were measured (SOF measured by Yanowitz and co-workers [1999a] and EC measured on the same samples by Zielinska and co-workers [1998]); a parity plot of these results is shown in Figure 2-21. The data reported by Zielinska and co-workers currently appear to be the only measurements of EC for in-use vehicles. These data are for 4-stroke engines. EC ranged from 31% to 84% of PM. and averaged 63%. It is apparent from Figure 2-20 that g/mile EC emissions have declined with model year.

Engine testing studies show SOF percentage to be highly variable, ranging from 20% to 60%, and exhibiting a declining trend with model year (McCarthy et al., 1992; Springer, 1979; Johnson et al., 1994; Bagley et al., 1998; Tanaka et al., 1998; Rantanen et al., 1993; Mitchell et al., 1994; Hansen et al., 1994). On a g/bhp-h basis SOF emissions declined significantly in the early 1990s and are typically in the range of 0.02-0.05 g/bhp-h. Engine dynamometer data provide confirmation that total SOF and PM solids (or EC) emissions have declined. Note that there are many more engine testing studies available, which this document does not attempt to comprehensively review.

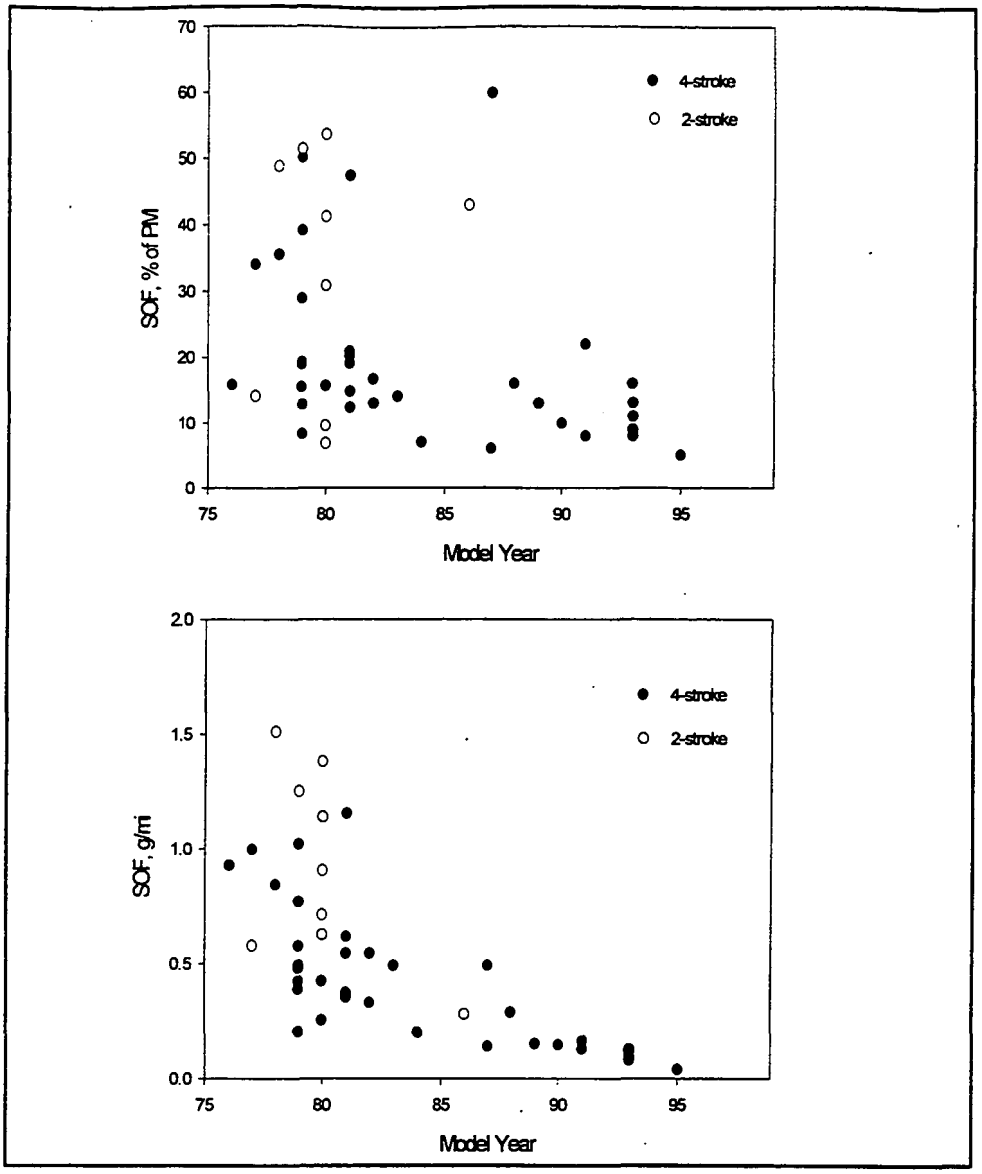


Figure 2-19. Comparison of SOF emissions for 2- and 4-stroke engines in g/mi and as a percentage of total PM.
Sources: Warner-Selph et al., 1984; Dietzmann et al., 1980; Graboski et al., 1998b.

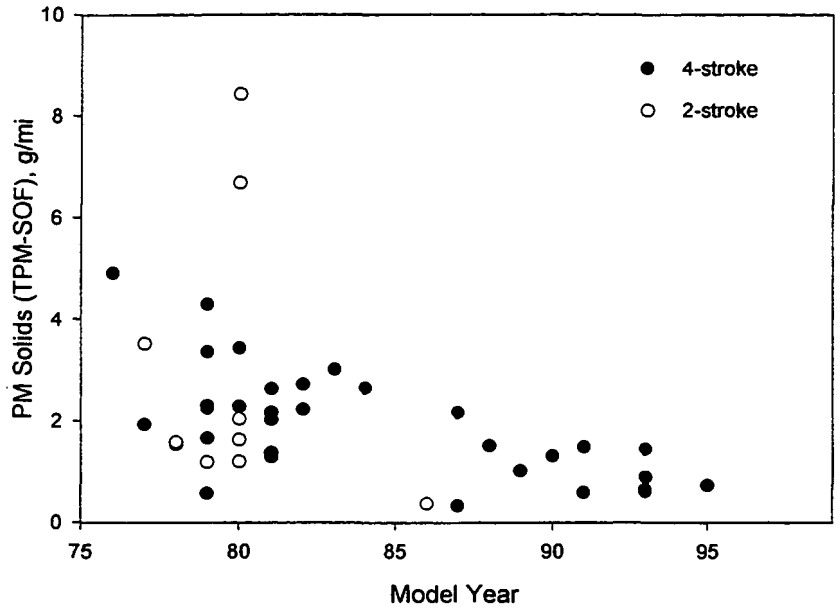


Figure 2-20. Trends in PM solids emissions with model year, a reasonable surrogate for elemental carbon content.
Sources: Yanowitz et al., 1999a; Warner-Selph and Dietzmann, 1984; Dietzmann et al., 1980; Rogge et al., 1993.

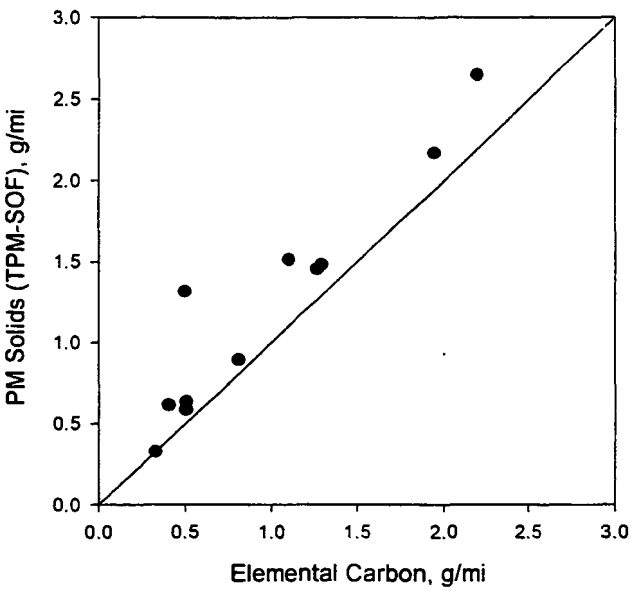


Figure 2-21. Parity plot showing approximate agreement between PM elemental carbon and PM solids measurements in g/mi.
Sources: Yanowitz et al., 1999a; Zielinska et al., 1998.

2.2.6.2. *PAHs and Nitro-PAH Emissions*

PAHs, nitro-PAHs, and oxidized derivatives of these compounds have attracted considerable attention because of their known mutagenic and, in some cases, carcinogenic character. Nitrated polycyclic aromatic compounds have caused lung cancer and remote metastases in laboratory animals (HEI, 1995). 1-Nitropyrene has been speculated to be the major source of mutagenicity in diesel soot (Noorkhoek and Bos, 1995); however, a large number of other nitro-PAHs are present (Paputa-Peck et al., 1983) and other studies suggest that it is the oxygenated nitro-PAH species that are responsible (Schuetzle et al., 1981; Schuetzle et al., 1985; Ciccioli et al., 1986). For example, Grosovsky and co-workers (1999) have shown that 2-nitrodibenzopyranone (2NDBP) is highly mutagenic in human cells. 3-Nitrobenzanthrone has been shown to be one of the most potent bacterial mutagens known (Enya et al., 1997). 3-Nitrobenzanthrone is also known to be a component of diesel PM, while 2NDBP is proposed to be an atmospheric transformation product of phenanthrene, but may also be present in diesel exhaust.

A few engine and chassis studies have measured PAH emissions. Dietzmann and co-workers (1980) examined four vehicles equipped with late 1970s turbocharged DI engines. Emissions of benzo(a)pyrene from particle extracts were reported and ranged from 1.5 to 9 μg/mi. No gas-phase PAH measurements were reported. No correlation with engine technology (one of the engines was 2-stroke) was observed. Table 2-7 gives the approximate concentrations of several of the abundant nitro-PAHs quantified in early 1980s LD-diesel particulate extracts (with the exception of 3-nitrobenzanthrone, reported recently), in μg/g of particles. Concentrations for some of the nitro-PAHs identified range from 0.3 μg/g for 1,3-dinitropyrene to 8.6 μg/g for 2,7-dinitro-9-fluorenone and 75 μg/g for 1-nitropyrene. More recent nitro-PAH and PAH data for HD diesel engines are reported in units of g/hgp-hr or mass/volume of exhaust,making it impossible to compare them to the older data (Norbeck et al., 1998; Bagley et al., 1996, 1998; Baumgard and Johnson, 1992; Opris et al., 1993; Hansen et al., 1994; Harvey et al., 1994; Kantola et al., 1992; Kreso et al., 1998b; McClure et al., 1992; Pataky et al., 1994).

Rogge and co-workers (1993) reported PAH emissions from particle extracts for two 1987 model year trucks (averaged together, 4-stroke and turbocharged engines). They report results for many specific PAH compounds with total PAHs summing to 0.43 mg/mi and benzo(a)pyrene emissions of 2.7 μg/mi. Particle-phase PAH was about 0.5% of total PM mass. Schauer and co-workers (1999) have recently reported gas- and particle-phase PAH emissions for a 1995 medium-duty turbocharged and intercooled truck. They also report results for a large number of individual PAHs, but summed emissions were 6.9 mg/mi (gas phase) and 1.9 mg/mi (particle phase). Particle-phase PAHs were about 0.7% of total PM mass. Emissions of benzo(a)pyrene were not reported, but emissions of individual species of similar molecular

Table 2-7. Concentrations of nitro-polycyclic aromatic hydrocarbons identified in a LD diesel 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-l-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
3-nitrobenzanthrone[d]	0.6 to 6.6

[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.
[d]From Enya et al., 1997 (Isuzu Model 6HEL 7127cc).

weight were approximately 10 μg/mi. Measurements of particle- and gas-phase PAHs conducted for the Northern Front Range Air Quality Study (Zielinska et al., 1998) found the benzo(a)pyrene emission rate to average 13 μg/mi for 15 vehicles ranging from 1983-1993 model years. Summing of individual PAH emissions from this study yields a total PAH rate (combined gas and particle phase) of 13.5 mg/mi.

Benzo(a)pyrene emissions were also reported in the engine dynamometer studies of Springer (1979). A comparison of turbocharged and naturally aspirated engines (both about 1 μg/bhp-h), and of DI and IDI engines (both about 0.15 μg/bhp-h) showed no significant effect of these technology changes on emissions of this compound, as shown in Table 2-8. The difference between 1 and 0.15 μg/bhp-h cannot be attributed to specific technology changes. The engines were from different manufacturers. Bagley and co-workers (1998) studied a 1983 model year IDI, naturally aspirated engine and observed emission levels listed in Table 2-8 from particulate matter extracts. These results can be compared to data presented by Mitchell and co-workers (1994) for two DI turbocharged engines. It is likely that there are also other differences in technology between the 1983 and 1991 engines; however, it is clear that total PM emissions are substantially lower for the newer engines. Results for SOF are inconclusive. Total PAH emissions are the same for the 1983 and 1991 engines and range from 0.05% to 0.15% of the total PM mass. 1-Nitropyrene emissions are near to detection limits and thus the apparent differences are probably not significant.

On the basis of these limited data it is difficult to draw a precise, quantitative conclusion regarding how PAH emissions have changed over time. However, it seems likely that total PAH emissions have been in the range from 0.1 to 15 mg/mi from the early 1970s to the early 1990s. PM-associated PAHs make up on the order of 0.1% of PM mass. Emissions of benzo(a)pyrene were on the order of 10 μg/mi. It is also highly likely that PAH emissions have declined in parallel with emissions of total PM and SOF, which have declined by a factor of approximately 6 over this time period. There is no evidence for a change in PAH emissions out of proportion to the observed changes in mass emissions of PM or SOF.

One chassis study has reported emissions of 1-nitropyrene in PM extracts from 17 HD diesel vehicles (Warner-Selph and Dietzmann, 1984). All engines were turbocharged and direct injected. Results are shown in Figure 2-22; unfortunately there are no distinct trends in these data and the data do not extend to the period of strict emission regulations in the late 1980s. The results suggest, however, that the introduction of new technologies, which did occur to some extent over the model years covered, has not produced dramatic changes in emissions of 1-nitropyrene. Again, it seems likely that nitro-PAH emissions have declined in parallel with decreasing emissions of total PM and SOF.

Table 2-8. Comparison of PAH and nitro-PAH emissions for IDI naturally aspirated engines and two DI turbocharged engines

Emissions, µg/bhp-h	1977 Mack ETAY(B)673A DI, turbo-charged, aftercooled	1977 Caterpillar 3206, DI, turbocharged, aftercooled	1977 Caterpillar 3206, IDI, turbocharged, aftercooled	1977 Daimler-Benz OM-352A, DI, turbo-charged, aftercooled	1977 Daimler-Benz OM-352A, DI, naturally aspirated	1983 Caterpillar 3304 IDI, naturally aspirated	1991 DDC Series 60 DI, turbocharged, aftercooled	1991 Navistar DTA466, DI, turbocharged, aftercooled
PM (g/bhp-h)	0.61	.35	0.28	0.56	0.99	0.56	0.12	0.1
SOF (%)	16	18	11	34	29	57	26	55
PAH	--	--	--	--	--	132.5	131	145
Benzopyrene	2.23	0.15	0.10	0.87	1.07	1.5	--	0.05
Nitro-PAH	--	--	--	--	--	--	0.18	0.65
1-Nitro-pyrene	--	--	-	-	-	2.2	0.06	0.32

Sources: Springer, 1979; Bagley et al., 1998; Mitchell et al., 1994.

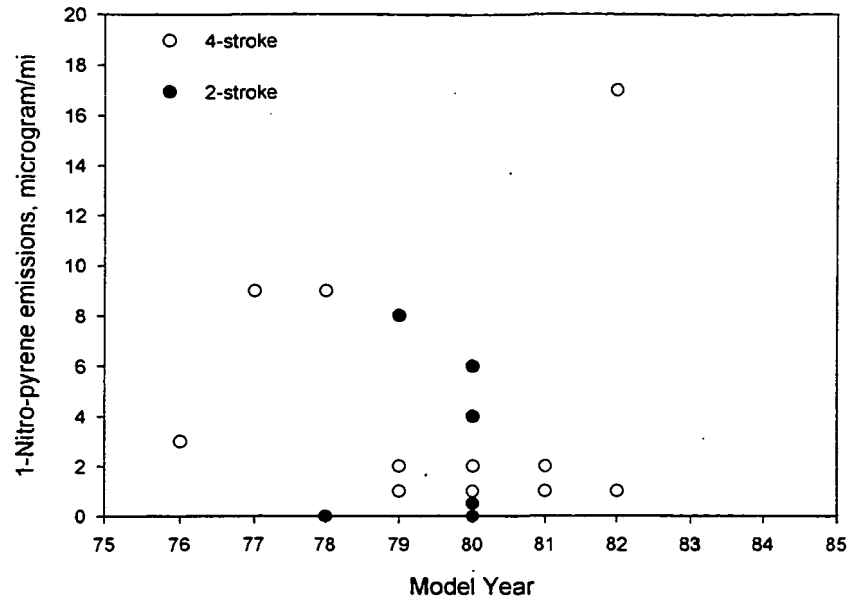


Figure 2-22. 1-Nitropyrene emission rates from several HD diesel vehicles.
Source: Warner-Selph et al., 1984.

2.2.6.3. *Aldehyde Emissions*

Many aldehydes of interest typically occur in the gas phase rather than the particle phase of diesel exhaust. Some aldehydes are also known carcinogens and there are considerable data on aldehyde emissions from diesel engines. Figure 2-23 reports mg/mile total aldehyde emissions from chassis dynamometer studies (Warner-Selph and Dietzmann, 1984; Schauer et al., 1999; Unnasch et al., 1993). The results indicate no difference between 2 and 4-stroke engines, although aldehyde emissions appear to have declined substantially since 1980 on the basis of a limited number of data points (only 2). Engine dynamometer studies show aldehyde emission levels of 150-300 mg/bhp-h for late 1970s engines with no significant effect of turbocharging, or IDI versus DI. High-pressure fuel injection may have resulted in a marginal increase in aldehyde emissions (Springer, 1979). By comparison, 1991 model year engines (DI, turbocharged) exhibited aldehyde emissions in the 30-50 mg/bhp-h range (Mitchell et al., 1994). It seems likely that aldehyde emissions have declined by perhaps one order of magnitude since about 1980, on average, in line with the decline in total PM and SOF emissions. Insufficient information is available to determine the cause of this decline; however, more complete combustion because of higher pressure fuel injection coupled with leaner operation because of turbocharging with aftercooling is the most likely cause.

2.2.6.4. *Dioxin and Furan Emissions*

Dioxin and furan emissions from on-road HD diesel vehicles were measured in the Fort McHenry Tunnel in Baltimore, MD (Gertler et al., 1998). For the limited range of vehicle operating conditions in the tunnel, the average HD diesel emission factor was 0.28±0.13 ng-TEQ/mi. This is a factor of 3 lower than the initial EPA estimate (Gertler et al., 1998). The recent dynamometer measurements from a HD diesel engine (Cummins L10) showed negligible dioxin and furan emission rates (Norbeck et al., 1998).

2.2.6.5. *Particle Size*

Figure 2-24 shows the general size distribution for diesel particulate based on mass and particle number. Most of the mass is accumulation mode particles ranging in size from 50 to 700 nm and averaging about 200 nm. Aggregated carbonaceous particles and absorbed organic material are primarily in this mode. The nuclei mode consists of particles in the 5-50 nm range, averaging about 20 nm. These are believed to form from exhaust constituents during cooling and to consist of sulfuric acid droplets, ash particles, condensed organic material, and perhaps primary carbon spherules (Abdul-Khalek et al., 1998; Baumgard and Johnson, 1996). The nuclei mode typically contains from 1%-20% of particle mass and from 50%-90% of the particle number.

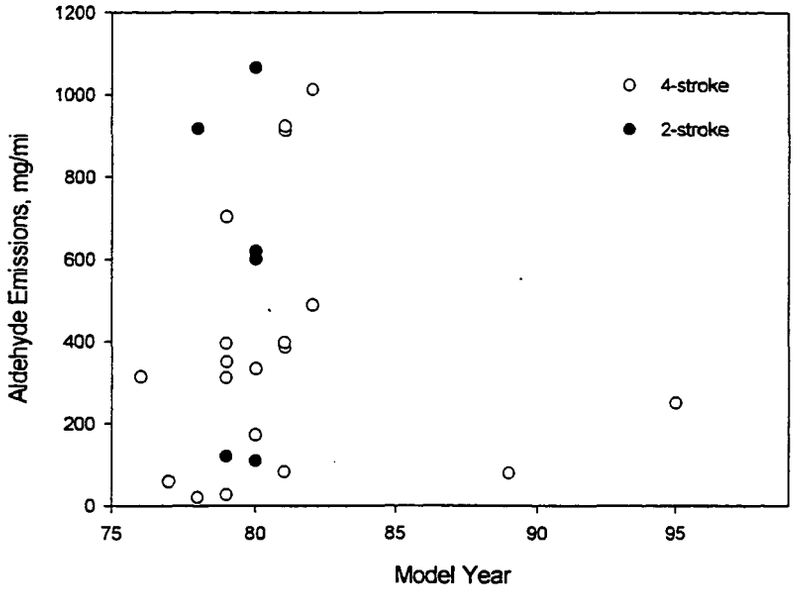


Figure 2-23. Chassis dynamometer measurements of total aldehyde emissions from HD diesel vehicles.
Sources: Warner-Selph and Dietzmann, 1984; Schauer et al., 1999; Unnasch et al., 1993.

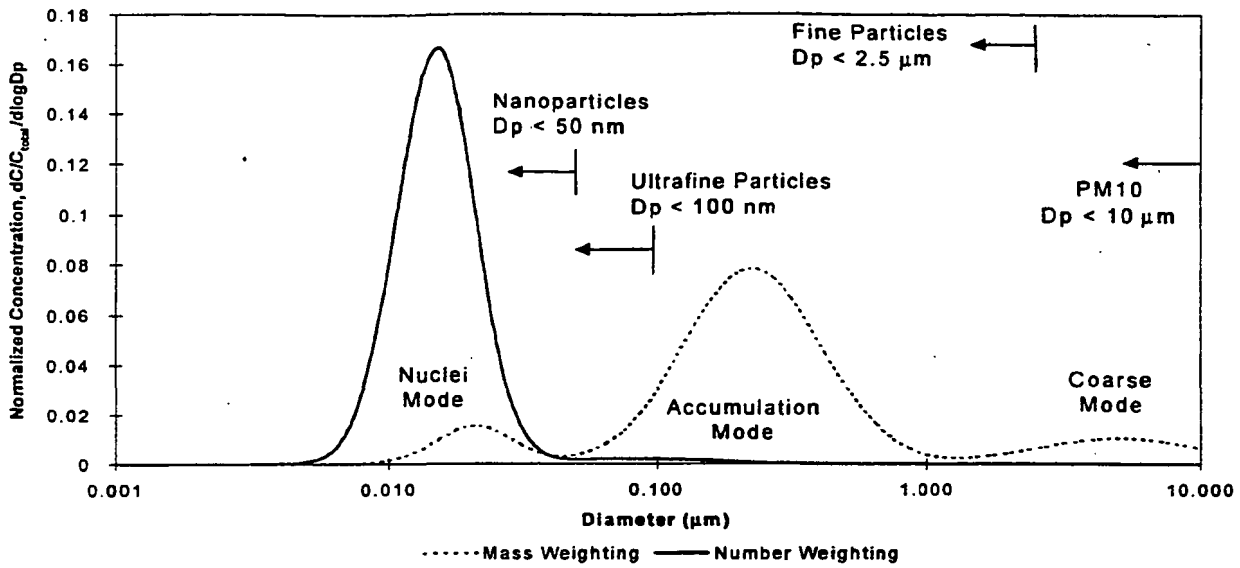


Figure 2-24. Particle size distribution in diesel exhaust, taken from Kittelson (1998).

Measurements made on diluted diesel exhaust typically show higher numbers of nuclei-mode particles than do measurements made on raw exhaust because of condensation to form nuclei mode aerosol upon cooling of the exhaust. Dilution ratio, sampling temperature, and other sampling factors can therefore have a large impact on the number and makeup of nuclei mode particles (Abdul-Khalek et al., 1999). Just as diesel exhaust particulate matter is defined by how it is collected (i.e., on a filter at or below 52 °C); the size distribution of diesel exhaust is also determined by how is it measured. Baumgard and Johnson (1996) have proposed that accumulation-mode particles are formed in the combustion chamber whereas nuclei-mode particles are formed during the dilution and measurement process. It seems likely that the situation is not nearly so clear-cut and that both accumulation and nuclei-mode particles are formed in the combustion chamber, but that a large number of additional nuclei-mode particles are formed during dilution.

Several groups have shown that decreasing sulfur content decreases the number of nuclei-mode particles measured in the exhaust, assuming temperature is low enough and residence time is long enough for nucleation and condensation of sulfate aerosol and water (Baumgard and Johnson, 1992; Opris et al., 1993; Baumgard and Johnson, 1996; Abdul-Khalek et al., 1999).

The application of this finding to real-world conditions is difficult to predict, as the number of nuclei-mode particles formed from sulfate and water in the atmosphere will be determined by atmospheric conditions, not by dilution tunnel conditions.

More controversial is the suggestion that PM emission size distribution from newer technology engines (1991 and later) may be shifted to have a much higher number concentration of nuclei-mode particles, independent of fuel sulfur content (Kreso et al., 1998b; Abdul-Khalek et al., 1998; Baumgard and Johnson, 1996; Bagley et al., 1996). For example, in the study of Kreso and coworkers (1998b), a comparison of emissions from a 1995 model year engine measured in that work with measurements made on 1991 (Bagley et al., 1996) and 1988 (Bagley et al., 1993) model year engines in earlier studies is presented. Dilution conditions (relatively low temperature, low primary dilution ratio, long residence time of more than 3 seconds) strongly favor the formation of nucleation products. The 1991 and 1988 engines were tested with 100 ppm sulfur fuel while the 1995 engine was tested with 310 ppm sulfur fuel, which may confound the results to some extent. Nuclei-mode particles made up 40% to 60% of the number fraction of PM emissions for the 1988 engine and 97%+ of the PM from the 1991 and 1995 engines. Number concentrations were also roughly two orders of magnitude higher for the newer engines. SOF made up 25%-30% of PM in the 1988 engine and 40%-80% of PM for the newer engines. Total PM was significantly reduced for the newer engines. It was suggested that increased fuel injection pressure leads to improved fuel atomization and evaporation, leading to smaller primary carbonaceous particles, but there appears to be no more direct experimental or computational evidence supporting this hypothesis. The high degree of SOF with the 1991 engine, particularly at high-load test modes, was also inconsistent with measured SOF values of other engines using similar types of technology (Last et al., 1995, Ullman et al., 1995). Kittelson (1998) notes that there is far less soot-type particulate and that higher number concentrations of the small particles are formed from nucleation of VOC and sulfuric acid-type compounds.

At present, no conclusions can be made regarding the reported shift in size distribution because:

- The result may simply be a sampling artifact because of the substantial effect of dilution conditions on measured particle size distributions. The results may also be a sampling artifact because no study has reported back-to-back testing of engines with varying technology. All results are based on a comparison of results from individual studies performed over several years. Only recently has the impact of sampling conditions begun to be understood, and thus early results, and results that are not clearly obtained under identical sampling conditions, may lead to erroneous conclusions. Extensive research is underway to understand the factors in the

sampling procedure that affect the PM size distribution and to determine the actual size distribution (Kittelson et al., 1999).

- The result may not be relevant to the fate of engine exhaust in the atmosphere. It is unclear what sampling conditions are appropriate for these studies. If understanding the fate of the exhaust in the atmosphere is the intended use of the study, then simulation of atmospheric dilution conditions is desirable. On the other hand, some studies may be more interested in the impact of engine technology on engine emissions with no sampling artifacts. Given an understanding of the atmospheric chemistry and physics, engine emissions might also be used to predict the formation of aerosol in the atmosphere. An understanding of these factors can only come through knowledge of the chemical composition of nuclei-mode particles and through studies of the fate of diesel exhaust in the atmosphere (an alarmingly complex situation).

- Particle sizing studies have been performed under steady-state conditions that are probably not representative of how nearly all diesel particulate is actually formed in use. Engine transients create temporary situations that favor PM production, and in all likelihood more than 90% of diesel PM in use is generated under these conditions.

2.3. ATMOSPHERIC TRANSFORMATION OF DIESEL EXHAUST

Primary diesel emissions are a complex mixture containing hundreds of organic and inorganic constituents in the gas and particle phases, the most abundant of which are listed in Table 2-9. The more reactive compounds with short atmospheric lifetimes will undergo rapid transformation in the presence of the appropriate reactants, whereas more stable pollutants can be transported over greater distances. A knowledge of the atmospheric transformations of gaseous and particulate components of diesel emissions and their fate is important in assessing environmental exposures and risks. This section describes some of the major atmospheric transformation processes for gas-phase and particle-phase diesel exhaust, focusing on the primary and secondary organic compounds that are of significance for human health. For a more comprehensive summary of the atmospheric transport and transformation of diesel emissions see Winer and Busby (1995).

Table 2-9. Classes of compounds in diesel exhaust

Particulate phase		Gas phase	
Heterocyclics, hydrocarbons (C_{14}-C_{35}), and PAHs and derivatives:		Heterocyclics, hydrocarbons (C_1-C_{10}), and derivatives:	
Acids	Cycloalkanes	Acids	Cycloalkanes, Cycloakenes
Alcohols	Esters	Aldehydes	Dicarbonyls
Alkanoic acids	Halogenated cmpds.	Alkanoic acids	Ethyne
n-Alkanes	Ketones	n-Alkanes	Halogenated cmpds.
Anhydrides	Nitrated cmpds.	n-Alkenes	Ketones
Aromatic acids	Sulfonates	Anhydrides	Nitrated cmpds.
	Quinones	Aromatic acids	Sulfonates
			Quinones
Elemental carbon		Acrolein	
Inorganic sulfates and nitrates		Ammonia	
Metals		Carbon dioxide, carbon monoxide	
Water		Benzene	
		1,3-Butadiene	
		Formaldehyde	
		Formic acid	
		Hydrogen cyanide, hydrogen sulfide	
		Methane, methanol	
		Nitric and nitrous acids	
		Nitrogen oxides, nitrous oxide	
		Sulfur dioxide	
		Toluene	
		Water	

Source: Mauderly (1992), which summarized the work of Lies et al., 1986; Schuetzle and Frazier, 1986; Carey 1987; Zaebst et al., 1988; updated from recent work by Johnson, 1993; McDonald, 1997; Schauer et al., 1999.

2.3.1. Gas-Phase Diesel Exhaust

Gas-phase diesel exhaust contains of several organic and inorganic compounds which undergo various chemical and physical transformations in the atmosphere depending on the abundance of reactants and meteorological factors such as wind speed and direction, solar irradiance, humidity, temperature, and precipitation. Gaseous diesel exhaust will primarily react with the following species (Atkinson, 1988):

- Sunlight, during daylight hours;
- Hydroxyl radical (OH), during daylight hours;
- Ozone (O_3), during daytime and nighttime;
- Hydroperoxyl radical HO_2, typically during afternoon/evening hours;
- Gaseous nitrate radicals (NO_3) or dinitrogen pentoxide (N_2O_5), during nighttime hours; and
- Gaseous nitric acid (HNO_3) and other species such as nitrous acid (HONO) and sulfuric acid (H_2SO_4).

The major loss process for most of the diesel exhaust emission constituents is oxidation, which occurs primarily by daytime reaction with OH radical (Table 2-10). For some pollutants, photolysis, reaction with ozone, and reactions with NO_3 radicals during nighttime hours are also important removal processes. 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 NO_3 appears to be of minor significance as a PAH loss process, but is more important as a route of formation of mutagenic nitro-PAHs. The reaction products for some of the major gaseous diesel exhaust compounds are listed in Table 2-11 and are discussed briefly below.

2.3.1.1. *Organic Compounds*

The organic fraction of diesel exhaust is a complex mixture of compounds, very few of which have been characterized. The atmospheric chemistry of several organic constituents of diesel exhaust (which are also produced by other combustion sources) has been studied. A few of these reactions and their products are discussed below. For a complete summary of the atmospheric chemistry of organic combustion products, see Seinfeld and Pandis (1998).

Acetaldehyde forms peroxyacetyl nitrate (PAN), which has been shown to be a direct-acting mutagen toward *S. typhimurium* strain TA100 (Kleindienst et al., 1985) and is phytotoxic. Benzaldehyde, the simplest aromatic aldehyde, forms peroxybenzoyl nitrate or nitrophenols following reaction with oxides of nitrogen (Table 2-11).

For those PAHs present in the gas phase, reaction with the hydroxyl radical is the major removal route, leading to atmospheric lifetimes of a few hours. The gas-phase reaction of PAHs containing a cyclopenta-fused ring, such as acenaphthene, acenaphthylene, and acephenanthrylene with the nitrate radical may be an important loss process during nighttime hours. Relatively few data are available concerning the products of these gas-phase reactions. It has 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 et al., 1986; Atkinson et al., 1986; 1990; Zielinska et al., 1988; 1989a; Arey, 1998). In addition, in a 2-step process involving OH radical reaction and NO_2 addition, 2-nitrofluoranthene and 2-nitropyrene can be formed and eventually partition to the particle phase, as will other nitro-PAHs.

The addition of the NO_3 radical to the PAH aromatic ring leads to nitroarene formation (Sweetman et al., 1986; Atkinson et al., 1987, 1990; Zielinska et al., 1989a). The gas-phase reactions of NO_3 radical with naphthalene, 1- and 2-methylnaphthalene, acenaphthene, phenanthrene, anthracene, fluoranthene, and pyrene produce, in general, the same nitro-PAH isomers as the OH radical reaction, but with different yields (Arey et al., 1989; Sweetman et al.,

Table 2-10. Calculated atmospheric lifetimes for gas-phase reactions of selected compounds present in automotive emissions with 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	1.3 days	12 h	24 min	2 h	2 min
NO	2.5 days	1 min	1.2 min	20 min	-
HNO_3	110 days	-	-	-	-
SO_2	16 days	>200 years	$>1.4\times10^{4}$ years	>600 years	-
NH_3	90 days	-	-	-	-
Propane	12 days	>7000 years	-	-	-
n-Butane	5.6 days	>4500 years	3.6 years	-	-
n-Octane	1.9 days	-	1.2 years	-	-
Ethylene	1.9 days	9 days	1.2 years	-	-
Propylene	7 h	1.5 days	6 days	-	-
Acetylene	19 days	6 years	>5.6 years	-	-
Formaldehyde	1.9 days	>2 - 104 years	84 days	23 days	4 h
Acetaldehyde	0.6 day	>7 years	20 days	-	60 h
Benzaldehyde	1.2 days	-	24 days	-	-
Acrolein	0.6 day	60 days	-	-	-
Formic acid	31 days	-	-	-	-
Benzene	11 days	600 years	>6.4 years	-	-
Toluene	2.5 days	300 years	3.6 years	-	-
m-Xylene	7 h	75 years	0.8 years	-	-
Phenol	6 h	-	8 min	-	-
Naphthalene[f]	6.8 h	>80 days	1.5 years	-	-
2-Methylnaphthalene[f]	2.8 h	>40 days	180 days		-
1-Nitronaphthalene[f]	2.3 days	>28 days	1 8 years		1.7 h
Acenaphthene[f]	1.5 h	>30 days	1.2 h	-	-
Acenaphthylene[f]	1.3 h	~43 min	6 min	-	-
Phenanthrene[f]	11.2 h	41 days	4.6 h	-	-
Anthracene[f]	8.6 h	-	-	-	-
Fluoranthene[f]	~2.9 h	-	~1 year	-	-
Pyrene[f]	~2.9 h	-	~ 120 days	-	-

[a] For 12-h average concentration of OH radical of 1.6×10^{6} molecule/cm^3 (Prinn et al., 1992).
[b] For 24-h average O_3 concentration of 7×10^{11} molecule/cm^3.
[c] For 12-h average NO_3 concentration of 5×10^{8} molecule/cm^3 (Atkinson, 1991).
[d] For 12-h average HO_2 concentration of 10^{8} molecule/cm^3.
[e] For solar zenith angle of 0°.
[f] Lifetimes from Arey (1998), for 12-h concentration of OH radical of 1.9×10^{6} molecule/cm^3.

Source: Winer and Busby (1995) unless noted otherwise.

Table 2-11. Major components of gas-phase diesel engine emissions and their known atmospheric transformation products

Emission component	Atmospheric reaction products
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., acetaldehyde, acrolein)	Peroxyacyl nitrates
Monocyclic aromatic compounds (e.g., benzene, toluene)	Hydroxylated and hydroxylated-nitro derivatives[a]
PAHs (≤4 rings) (e.g., phenanthrene, fluoranthene)[b]	Nitro-PAHs (4 rings)[c]
Nitro-PAHs (2 and 3 rings) (e.g., nitronaphthalenes)	Quinones and hydroxylated-nitro derivatives

[a] Some reaction products expected to partition into the particle phase.
[b]Nitro-PAHs with more than two rings will partition into the particle phase.
[c]PAHs containing four rings are usually present in both the vapor and particle phases.

Source: Adapted from Winer and Busby, 1995.

1986; Atkinson et al., 1987, 1990; Zielinska et al., 1986, 1989a). For example, the same 2-NF is produced from both OH radical and NO_3 gas-phase reactions, but the reaction with NO_3 produces a much higher yield. While the production of several nitroarene compounds has been studied in environmental chambers (Arey et al., 1989; Zielinska et al., 1990; Atkinson and Arey, 1994; Arey, 1998; Feilberg et al., 1999), generally the same nitro-PAH isomers formed from reaction with OH and NO_3 radicals are observed in ambient air samples. Secondary formation of nitroarenes through the gas-phase reactions of the 2-, 3-, and 4-ring PAHs is the major source for many of the nitroarenes observed in ambient air (Pitts et al., 1985a,b,c; Arey et al., 1986; Zielinska et al., 1988). Photolysis is the major removal pathway for nitroarenes with lifetimes of approximately 2 hours (Feilberg et al., 1999; Nielsen and Ramdahl, 1986).

2.3.1.2. *Inorganic Compounds*

Sulfur dioxide (SO_2) and oxides of nitrogen (primarily NO) are emitted from diesel engines. Sulfur dioxide is readily oxidized by the OH radical in the atmosphere, followed by formation of the HO_2 radical and HSO_3, which rapidly reacts with water to form sulfuric acid (H_2SO_4) aerosols. Because SO_2 is soluble in water, it is scavenged by fog, cloud water, and raindrops. In

aqueous systems, SO_2 is readily oxidized to sulfate by reaction with H_2O_2, O_3, or O_2 in the presence of a metal catalyst (Calvert and Stockwell, 1983). Sulfur emitted from diesel engines is predominantly (~98%) in the form of SO_2, a portion of which will form sulfate aerosols by the reaction described above. Off-road equipment, which typically uses fuel containing 3300 ppm sulfate, emits more SO_2 than on-road diesel engines, which use fuels currently containing an average of 340 ppm sulfur because of EPA regulations effective in 1993 decreasing diesel fuel sulfur levels. EPA (1998b) estimates that mobile sources are responsible for about 7% of nationwide SO_2 emissions, with diesel engines contributing 80% of the mobile source total (the majority of the diesel SO_2 emissions originate from nonroad engines) (U.S. EPA, 1998b).

Nitric oxide (NO) is also oxidized in the atmosphere to form NO_2 and particulate nitrate. The fraction of motor vehicle NO_x exhaust converted to particulate nitrate in a 24-hour period has been calculated using a box model to be approximately 3.5% nationwide, a portion of which can be attributed to diesel exhaust (Gray and Kuklin, 1996). EPA estimates that in 1997, mobile sources were responsible for about 50% of nationwide NO_x emissions, with diesel engines being responsible for approximately half of the mobile source total (U.S. EPA, 1998b).

2.3.1.3. *Atmospheric Transport of Gas-Phase Diesel Exhaust*

Gas-phase diesel exhaust can be dry deposited, depending on the deposition surface, atmospheric stability, and the solubility and other chemical properties of the compound. Dry deposition of organic species is typically on the order of weeks to months, with dry deposition velocities of approximately 10^{-4} cm/sec (Winer and Busby, 1995). In contrast, inorganic species such as sulfur dioxide and nitric acid have relatively fast deposition rates (0.1-2.5 cm/sec) and will remain in the atmosphere for shorter time periods compared with the organic exhaust components. Some gas-phase species will also be scavenged by aqueous aerosols and potentially deposited via precipitation. These processes can greatly reduce the atmospheric concentration of some vapor-phase species. Atmospheric lifetimes for several gas-phase components of diesel exhaust are on the order of hours or days, during which time atmospheric turbulence and advection can disperse these pollutants widely

2.3.2. Particle-Phase Diesel Exhaust

Particle-associated diesel exhaust is composed of primarily carbonaceous material (organic and elemental carbon) with a very small fraction composed of inorganic compounds and metals. The organic carbon fraction adsorbed on diesel PM is composed of high-molecular-weight compounds, such as PAHs, which are generally more resistant to atmospheric reactions than PAHs in the gas phase. The elemental carbon component of diesel exhaust is inert to

atmospheric degradation, while the PAH compounds are degraded by reaction with the following species:

- Sunlight, during daylight hours;
- Ozone (O_3), during daytime and nighttime;
- Nitrate radical (NO_3) and dinitrogen pentoxide (N_2O_5), during nighttime hours;
- Hydroxyl (OH) and hydroperoxyl radicals (HO_2);
- NO_2, during nighttime and daytime hours;
- Hydrogen peroxide (H_2O_2); and
- Gaseous nitric acid (HNO_3) and other species such as nitrous acid (HONO) and sulfuric acid (H_2SO_4).

Since many of the PAH derivatives formed by reaction with some of the reactants listed above have been found to be highly mutagenic, a brief discussion of PAH photolysis, nitration, and oxidation follows. Some of the major degradation products from particulate diesel exhaust are listed in Table 2-12.

Table 2-12. Major components of particle-phase diesel engine emissions and their known atmospheric transformation products

Emission component	Atmospheric reaction products
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

Source: Adapted from Winer and Busby, 1995.

2.3.2.1. *Particle-Associated PAH Photooxidation*

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 (Yokely et al., 1986; Dunstan et al., 1989) have shown that the extent of photodegradation of PAHs depends 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 appears that PAHs were stabilized if the carbon black content of the fly ash was greater than approximately 5%. On black substrates, half-lives of PAHs studied were on the order of several days (Behymer and Hites, 1988).

The environmental chamber studies of Kamens et al. (1988) on the daytime decay of PAHs present on residential wood smoke particles and on gasoline internal combustion emission particles showed PAH half-lives of approximately 1 hour 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. The presence and composition of an organic layer on the aerosol seems to influence the rate of PAH photolysis (Jang and McDow, 1995; McDow et al., 1994; Odum et al., 1994).

Because of 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, it is reasonable to speculate that PAHs adsorbed onto these particles might be relatively stable under standard atmospheric conditions, leading to an anticipated half-life of 1 or more days.

2.3.2.2. *Particle-Associated PAH Nitration*

Since 1978, when Pitts et al. (1978) first demonstrated that benzo(a)pyrene deposited on glass-fiber filters exposed to air containing 0.25 ppm NO_2 with traces of HNO_3 formed nitro-benzo(a)pyrene, numerous studies of the heterogeneous nitration reactions of PAHs adsorbed on a variety of substrates in different simulated atmospheres have been carried out (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 most abundant isomers formed were 1-nitropyrene (1-NP) from pyrene, 6-nitro-benzo(a)pyrene from benzo(a)pyrene, and 3-nitroperylene from perylene.

The formation of nitro-PAHs during sampling may be an important consideration for diesel PM collection because of the presence of NO_2 and HNO_3 (Feilberg et al., 1999). However, Schuetzle (1983) concluded that the artifact formation of 1-NP was less than 10%-20% of the 1-NP present in the diesel particles if the sampling time was less than 23 min (one FTP cycle) and

if the sampling temperature was 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).

Diesel PM 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 as 2.1 ppm and ~5 ppm by mass of the extractable material from diesel and SI vehicle PM, respectively. These values are much lower than would be predicted on the basis of laboratory measurements for either diesel or SI engines (Gorse et al., 1983).

Several nitroarene measurements have been conducted in airsheds heavily affected by motor vehicle emissions (Arey et al., 1987; Atkinson et al., 1988; Zielinska et al., 1989a,b; Ciccioli et al., 1989, 1993). Ambient PM samples were collected at three sites in the Los Angeles Basin during two summertime periods and one wintertime period. Concentrations of 1-NP ranged from 3 pg/m^3 to 60 pg/m^3 and 3-NF was also present in diesel PM at concentrations ranging from not detectable to 70 pg/m^3.

2.3.2.3. *Particle-Associated PAH Ozonolysis*

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

The reaction of PAHs 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 on the order of 0.5 to 1 hour have been reported for most PAHs studied (Van Vaeck and Van Cauwenberghe, 1984). The most reactive PAHs include benzo(a)pyrene, 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]perylene is less reactive than its isomer benzo[a]pyrene. The implications of this study for the high-volume sampling 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.

2.3.2.4. *Atmospheric Transport of Diesel Exhaust Particle Matter*

Ultrafine particles emitted by diesel engines undergo nucleation, coagulation, and condensation to form fine particles. PM can be removed from the atmosphere by dry and wet deposition. Particles of small diameter (<1 μm), such as diesel PM, are removed less efficiently than larger particles by wet and dry deposition and thus have longer atmospheric residence times. Dry deposition rates vary depending on the particle size. Because of their small size, diesel exhaust particles will have residence times of several days (dry deposition velocities of approximately 0.01 cm/sec) (Winer and Busby, 1995). Diesel particulates may be removed by wet deposition if they serve as condensation nuclei for water vapor deposition, or are scavenged by precipitation in- or below-cloud.

In a study designed to assess the atmospheric concentrations and transport of diesel exhaust particles, Horvath et al. (1988) doped the sole source of diesel fuel in Vienna with an organometallic compound of the heavy earth element dysprosium. The authors found that in some of the more remote sampling areas, diesel PM comprised more than 30% of the particulate mass, indicating that diesel PM can be dispersed widely.

2.3.3. Diesel Exhaust Aging

After emission from the tailpipe, diesel exhaust undergoes dilution, reaction, and transport in the atmosphere. The primary emission is considered "fresh," while "aged" diesel exhaust is considered to have undergone chemical and physical transformation and dispersion over a period of a day or two. Laboratory dilution tunnel measurements represent a homogeneous environment compared to the complex and dynamic system into which real-world diesel exhaust is emitted. The physical and chemical transformation of diesel exhaust will vary depending on the environment into which it is emitted. In an urban or industrial environment, diesel exhaust may enter an atmosphere with high concentrations of oxidizing and nitrating radicals, as well as nondiesel organic and inorganic compounds that may influence the toxicity, chemical stability, and atmospheric residence time. In general, secondary pollutants formed in an aged aerosol mass are more oxidized, and therefore have increased polarity and water solubility (Finlayson-Pitts and Pitts, 1986). These oxidized compounds may be removed at rates different from their precursor compounds and may exhibit different biological reactivities.

In addition, particle size distributions may vary depending on aggregation and coagulation phenomena in the aging process. People in vehicles, near roadways (e.g., cyclists, pedestrians, people in nearby buildings) and on motorcycles will be exposed to more fresh exhaust than the general population. In some settings where emissions are entrained for long periods through meteorological or other factors, exposures would be expected to include both fresh and aged diesel exhaust. The complexities of transport and dispersion of emission arising

from motor vehicles have been the subject of extensive modeling and experimental studies over the past decades and have been summarized by Sampson (1988); exposures to diesel PM are discussed in the next section of this chapter.

The major organic constituents of diesel exhaust and their potential degradation pathways described above provide evidence for (1) direct emission of PAHs, (2) formation of nitroarenes, and (3) secondary sulfate and nitrate formation. Since nitro-PAH products are often more mutagenic than their precursors, the formation, transport, and concentrations of these compounds in an aged aerosol mass are of significant interest.

2.4. AMBIENT DIESEL EXHAUST CONCENTRATIONS AND EXPOSURES

2.4.1. Diesel Exhaust Gases in the Ambient Atmosphere

Diesel exhaust gas is a complex mixture composed mainly of nitrogen, carbon dioxide, carbon monoxide, sulfur dioxide, and volatile organic compounds including aldehydes, alkanes, alkenes, and aromatic compounds such as benzene, toluene, 1,3-butadiene, naphthalene, and other low-molecular-weight aromatics. The primary source of these gas-phase compounds is incomplete fuel combustion and lubricating oil, with some contribution from compounds formed during the combustion process or by reaction with catalysts (Johnson et al., 1994). While direct emissions of several diesel exhaust components have been measured, few studies have attempted to elucidate the contribution of diesel-powered engines to atmospheric concentrations of these components, most of which are emitted by several combustion sources.

The emission profile of gaseous organic compounds is different for diesel and SI vehicles; the low-molecular-weight aromatic hydrocarbons and alkanes ($<C_9$) are more characteristic of SI engine emissions, whereas the heavier alkanes ($>C_{10}$) and aromatic hydrocarbons (such as naphthalene, methyl- and dimethyl- naphthalenes, methyl- and dimethyl-indans) are more characteristic of diesel engine emissions. These differences were the basis for apportionment of gasoline- and diesel-powered vehicle emissions to ambient nonmethane hydrocarbon (NMHC) concentrations in the Boston and Los Angeles (South Coast Air Basin) urban areas. The chemical mass balance receptor model (described below) was applied to ambient samples collected in these areas, along with appropriate fuel, stationary, and area source profiles (Fujita et al., 1997). The average of the sum of NMHC attributed to diesel exhaust, gasoline-vehicle exhaust, liquid gasoline, and gasoline vapor was 73% and 76% for Boston and the South Coast Air Basin (SoCAB), respectively. The average source contributions of diesel exhaust to NMHC concentrations were 22% and 13% for Boston and the SoCAB, respectively. The relative contribution of diesel exhaust will clearly depend on several factors including fleet composition, sampling location (e.g., near a bus station vs. near a highway or other sources), and the contribution from point and area sources. The source apportionment in the Fujita et al.

(1997) study indicates that mobile vehicle-related emissions account for the majority of ambient NMHC in the two urban areas studied and the results can likely be extrapolated to other urban areas with similar source compositions.

2.4.2. Ambient Concentrations of Diesel PM

The EPA Office of Air Quality Planning and Standards report on national air pollutant emission trends indicates that annual emissions of diesel PM less than 2.5 μm (PM2.5) nationwide are 5.7% of the total PM2.5 inventory (21% excluding natural and fugitive dust sources) (U.S. EPA, 1998b). The inventory includes on-road and off-road sources that have specific local and regional distributions. As a result of inhomogeneous source distributions, ambient diesel PM concentrations will vary by location. Only a few studies have been conducted to assess diesel PM concentrations in urban and rural areas, local "hotspots," and the potential for diesel PM episodes. The main approaches used to estimate the contribution of diesel exhaust to ambient PM concentrations are receptor modeling, elemental carbon surrogate calculations, and dispersion modeling. Studies conducted in Europe and Japan were reviewed, but, for the most part, were not included because of questions surrounding the applicability of measurements in locations that use different diesel technology and control measures from the United States.

2.4.2.1. *Receptor Modeling Estimates of Diesel PM*

Receptor models are used to infer the types and relative contributions of sources impacting a receptor site on the basis of measurements made at the receptor site for the pollutants of interest. As such, receptor models are referred to as "top-down" in contrast to "bottom-up" methods, which use emission inventory data, activity patterns, and dispersion modeling from the source to predict concentrations at a receptor site. Receptor models assume that the mass is conserved between the source and receptor site and that the measured mass of each pollutant is a linear sum of the contribution from each source.

The most commonly used receptor model for quantifying concentrations of diesel PM at a receptor site is the chemical mass balance model (CMB). Input to the CMB model includes PM measurements made at the receptor site as well as measurements made of each of the source types suspected to impact the site. Because of problems involving the elemental similarity between diesel and gasoline emission profiles and their co-emission in time and space, it is necessary to carefully quantify chemical molecular species that provide markers for separation of these sources (Lowenthal et al., 1992). Recent advances in chemical analytical techniques have facilitated the development of sophisticated molecular source profiles, including detailed speciation of organic compounds, which allow the apportionment of PM to gasoline and diesel

sources with increased certainty. Older studies that made use of only elemental source profiles have been published and are summarized here, but are subject to more uncertainty.

The CMB model has been used to assess the contribution of diesel PM to total PM mass in areas of California, Denver, Phoenix, and Manhattan (Table 2-13). Diesel PM concentrations reported by Schauer et al. (1996) for data collected in 1982 ranged from 4.4 $\mu g/m^3$ in west Los Angeles to 11.6 $\mu g/m^3$ in downtown Los Angeles. The average contribution of diesel PM to total PM mass ranged from 13% in Rubidoux to 36% in downtown Los Angeles. It should be noted that this model accounts for primary emissions of diesel PM only; the contribution of secondary aerosol formation (both acid and organic aerosols) is not included. In sites downwind from urban areas, such as Rubidoux in this study, secondary nitrate formation can account for a

Table 2-13. Ambient diesel PM concentrations reported from chemical mass balance modeling

Author	Year of sampling, no. days	Location	Location type	Source profile used	Total PM2.5 (stdev), $\mu g/m^3$	Diesel PM2.5 (stdev), $\mu g/m^3$
Schauer et al., 1996, Southern California	1982, 60 days (one every sixth day)	West LA Pasadena Rubidoux Downtown LA	Urban Urban Suburban Urban	OC species, EC, elements	24.5 (2.0) 28.2 (1.9) 42.1 (3.3) 32.5 (2.8)	4.4 (0.6) 5.3 (0.7) 5.4 (0.5) 11.6 (1.2)
Chow et al., 1991	Winter, 1989-90, NA	Phoenix, AZ area	Urban	NA	NA	4-22[a]
California EPA, 1998	1988-92, approx. 150 days	15 Air basins	Rural-urban	EC, OC total, Elements, Major Ions	NA	0.2-3.6[a]
Federal Highway Admin., 1997b	Spring, 1993, 3 days	Manhattan, NY	Urban bus stop	EC, OC total, elements, major ions	35.8-83.0	13.2-46.7[a]
NFRAQS, 1998	Winter, 1996-97, 60 days	Welby, CO Brighton, CO	Urban Suburban	OC Species, EC, elements, major ions	16.7 12.4	1.7 1.2

[a] PM10.
OC: Organic carbon.
EC: Elemental carbon.
NA: Not available.
Major ions: nitrate, sulfate, chloride and, in some cases, ammonium, sodium, potassium.

substantial fraction of the mass (25% of the fine mass measured in Rubidoux was attributed to secondary nitrate), a portion of which comes from diesel exhaust (Gray and Kuklin, 1996).

A wintertime study conducted in the Phoenix area by Chow et al. (1991) indicated that diesel PM levels on single days can range from 4 $\mu g/m^3$ in west and central Phoenix to 14 $\mu g/m^3$ in south Scottsdale and 22 $\mu g/m^3$ in central Phoenix. This apportionment, like the Schauer et al. (1996) data, reflects direct emissions only. These data relied on source profiles and ambient data collected prior to the introduction of technology to reduce PM emissions from diesel-powered vehicles.

A second CMB study reported ambient diesel PM concentrations for California and used ambient measurements from the San Joaquin Valley (1988-89), South Coast (1986), and San Jose (winters for 1991-92 and 1992-93) (California EPA, 1998a). The incorporation of sampling data from later dates provides information regarding exposures more relevant to current levels. The CMB in the California study (1998a) indicated that on an annual basis, basin-wide levels of direct diesel PM emissions may be as low as 0.2 $\mu g/m^3$ in the Great Basin Valleys and as high as 3.6 $\mu g/m^3$ in the South Coast basin.

The most recent study reporting diesel PM concentrations is from winter 1996-1997 sampling conducted in the Denver area as part of the Northern Front Range Air Quality Study (NFRAQS), (NRC, 1998). Ambient levels of diesel PM in the urban core site at Welby averaged 1.7 $\mu g/m^3$ over a 60-day winter period, and a slightly lower average concentration of 1.2 $\mu g/m^3$ was measured at an urban downwind site in Brighton, CO. One of the major findings from this study was a substantial contribution of elemental carbon from gasoline-powered vehicles. At the Welby site, the contribution of diesel and gasoline emissions to elemental carbon measurements was 52% and 42%, respectively. At the Brighton site, the contribution of diesel and gasoline emissions to elemental carbon measurements was 71% and 26%, respectively. The findings from the NFRAQS study are compelling and suggest the need for further investigations of this type that specifically address high-emitting vehicles. Geographical and other site-specific parameters that influence PM concentrations, such as altitude, must be considered when extrapolating the NFRAQS findings to other locations.

Limited data are available to allow a characterization of diesel PM concentrations in "hotspots" such as near heavily traveled roadways, bus stations, train stations, and marinas. One "hotspot" study conducted in Manhattan reported diesel PM concentrations of 13.0 to 46.7 $\mu g/m^3$ during a 3-day sampling period in the spring of 1993 (Federal Highway Administration, 1997b). This study attributed, on average, 50% of the PM to diesel exhaust. The diesel PM concentrations resulting from the source apportionment method used in this study require some caution. The CMB model overpredicted PM10 concentrations by an average 30%, suggesting that additional sources of the mass were not accounted for in the model. New advances in

organic carbon speciation, as has been noted above, are necessary to most appropriately characterize gasoline and diesel PM sources to ambient PM measurements. The relevance of the Manhattan bus stop exposure for large urban populations provides strong motivation for further studies in the vicinity of such "hotspots."

In summary, recent source apportionment studies (California EPA, 1998a; NRC, 1998) indicate that ambient diesel PM concentrations averaged over 2-12 month periods for urban/suburban areas can range from approximately 1.2 $\mu g/m^3$ to 3.6 $\mu g/m^3$, while diesel PM concentrations in more rural/remote areas are generally less than 1.0 $\mu g/m^3$. In the vicinity of "hotspots," or for short exposure times under episode-type conditions, diesel PM concentrations are expected to be substantially higher than these levels; however, a thorough and replicated characterization of these situations is not yet available. Two studies nearing completion by the South Coast Air Quality Management District will shed some light on near-highway concentrations of diesel PM (SCAQMD, 1999).

2.4.2.2. *Elemental Carbon Surrogate for Diesel PM*

Elemental carbon (EC) is a major component of diesel exhaust, contributing to approximately 60%-80% of diesel particulate mass, depending on engine technology, fuel type, duty cycle, lube oil consumption, and state of engine maintenance (Graboski et al., 1998; Zaebst et al., 1991; Pierson and Brachaczek, 1983; Warner-Selph et al., 1984). In most ambient environments, diesel PM is one of the major contributors to EC, with other potential sources including spark-engine exhaust; combustion of coal, oil, or wood; charbroiling; cigarette smoke; and road dust. Gasoline combustion was recently found to be an important source of elemental carbon in Denver (NRC, 1998).

Because of the large portion of EC in diesel PM, and the fact that diesel exhaust is one of the major contributors to EC in most ambient environments, diesel PM concentrations can be bounded using EC measurements. Source apportionment (NRC, 1998) indicates that diesel exhaust comprises from 52% to 71% of the elemental carbon concentrations in ambient PM in the Denver area for the winter of 1996-97. If we assume that gasoline and diesel exhaust contributions to EC measured in Denver will be similar to other areas, a plausible estimate of diesel particulate concentrations can be calculated by multiplying a measured EC concentration by 64% and dividing by the fraction of diesel PM mass accounted for by EC (note: a middle-of-the-range value of 70% was chosen for illustrative purposes), e.g., diesel PM concentration = [(EC * 0.64)/0.7]. This estimate uses an average of the diesel contributions to EC observed in Denver with contributions from other, potentially site-specific sources of EC subtracted (e.g., wood smoke). An upper-bound estimate of diesel PM from EC measurements would attribute all

ambient EC to diesel exhaust, e.g., diesel PM concentration = EC/0.7, which may be applicable to some occupational exposures.

The surrogate diesel PM calculation is a useful approach for estimating diesel PM in the absence of a more sophisticated receptor modeling analysis for locations where fine PM elemental carbon concentrations are available. Table 2-14 provides diesel PM concentrations that were calculated using the EC surrogate ratiometric approach. Under an EPA Research Grant with the Northeastern States for Coordinated Air Use Management (NESCAUM), PM2.5 samples were collected in Boston (Kenmore Square), MA, Rochester, NY, Reading, MA, Quabbin Reservoir, MA, and Brockport, NY (Salmon et al.. 1997). Samples were collected every sixth day for one year (1995). Using the EC surrogate calculation described above, diesel PM concentrations are estimated to range from 0.3 $\mu g/m^3$ in Brockport, NY to 1.1 $\mu g/m^3$ in Boston.

The Interagency Monitoring of Protected Visual Environments (IMPROVE) project of the National Park Service operates an extensive aerosol monitoring network in mainly rural or remote areas of the country (National Parks, National Monuments, Wilderness Areas, National Wildlife Refuges, and National Seashores). PM2.5 samples, collected twice weekly for 24-hour duration, at 43 sites (some co-located in the same rural park area) were analyzed for a suite of chemical constituents, including elemental carbon. The IMPROVE data in Table 2-14 represent average values for the period from March 1992 through February 1995 (Sisler, 1996).

Table 2-14. Diesel PM 2.5 concentrations in urban and rural locations using EC surrogate[a] for NESCAUM (1995) and IMPROVE (1992-1995) network sites

Area	Annual average PM2.5 $\mu g/m^3$	Diesel PM2.5 $\mu g/m^3$
Urban areas		
Boston, MA[b]	16.2	1.1
Rochester, NY[b]	14.9	0.5
Washington, DC[c]	19.2	1.6
Nonurban areas		
Quabbin, MA[b]	12.4	0.4
Reading, MA[b]	14.6	0.6
Brockport, NY[b]	12.8	0.3
IMPROVE sites	1.8-12.1	0.1-0.8

[a] Assumes 64% of EC mass is from diesel exhaust and 70% of diesel PM is elemental carbon.
[b] NESCAUM sites.
[c] IMPROVE sites.

The estimated diesel PM concentrations for rural/remote areas from the IMPROVE network ranged from 0.1 $\mu g/m^3$ for Denali National Park, AK, to 0.8 $\mu g/m^3$ for the Lake Tahoe, CA, area (which includes on-road and off-road diesel emissions). The diesel PM concentrations in rural areas of the northeastern States are similar to those calculated for rural areas in other parts of the country, with all values less than 1.0 $\mu g/m^3$. Among the urban areas included in the NESCAUM and IMPROVE networks, Washington, DC, had the highest calculated diesel PM concentration of 1.6 $\mu g/m^3$. The annual average value for Washington, DC, is quite similar to the wintertime diesel PM concentrations reported for Denver (NRC, 1998). Seasonally averaged data for the Washington, DC, site indicates that EC concentrations, and by extension, diesel PM concentrations at this site peak in the autumn and winter (1.9 and 1.8 $\mu g/m^3$ diesel PM, respectively).

Recently, EC measurements were reported for enclosed vehicles driving on Los Angeles roadways (California EPA, 1998b). Applying the ratiometric approach for diesel PM determinations from EC measurements, diesel PM concentrations in the vehicle ranged from approximately 2.8 $\mu g/m^3$ to 36.6 $\mu g/m^3$ depending on the type of vehicle being followed (higher concentrations were observed when the vehicle followed HD diesels). The California Air Resources Board also collected EC near the Long Beach Freeway for 4 days in May 1993 and 3 days in December 1993 (California EPA, 1998a). Using emission estimates from their EMFAC7G model, and elemental/organic carbon composition profiles for diesel and gasoline exhaust, tire wear, and road dust, the California Air Resources Board estimated the contribution of the freeway to diesel PM concentrations. For the 2 days of sampling in December 1993, diesel exhaust from vehicles on the nearby freeway were estimated to contribute from 0.7 $\mu g/m^3$ to 4.0 $\mu g/m^3$ excess diesel PM above background concentrations, with a maximum of 7.5 $\mu g/m^3$.

In two additional studies, EC concentrations were measured in Glendora, CA, during a carbonaceous aerosol intercomparison study (Cadle and Mulawa, 1990; Hansen and Novakov, 1990). EC concentrations ranged from 5.0 $\mu g/m^3$ to 6.4 $\mu g/m^3$, corresponding to diesel PM concentrations of 4.6 $\mu g/m^3$ to 5.9 $\mu g/m^3$ using the ratiometric approach described above. One technique used during the study reported EC concentrations in 1-minute intervals, revealing the impact from diesel vehicles 50 meters from the study site. The diesel vehicles were estimated to contribute up to 5 $\mu g/m^3$ EC above the background concentration.

In a study designed to investigate relationships between diesel exhaust exposure and respiratory health of children in the Netherlands, EC measurements were collected in 23 schools located from 47 to 377 meters from a freeway and in 8 schools located at a distance greater than 400 meters from a freeway (Brunekreef, 1999). EC concentrations in schools near freeways ranged from 1.1 to 6.3 $\mu g/m^3$, with a mean of 3.4 $\mu g/m^3$, and EC concentrations in schools more

than 400 meters from freeways ranged from 0.8 to 2.1 $\mu g/m^3$ with a mean of 1.4 $\mu g/m^3$. Using the EC surrogate calculation for diesel PM described above, the estimated average diesel PM concentration in the schools near a freeway is 3.1 $\mu g/m^3$ and the estimated average diesel PM concentration in schools located more than 400 meters from a freeway is 1.3 $\mu g/m^3$. Total PM2.5 mass inside the schools averaged 23.0 $\mu g/m^3$ while PM2.5 outside was only slightly higher (24.8 $\mu g/m^3$), suggesting extensive intrusion of outdoor air into the school environment.

2.4.2.3. *Dispersion Modeling Results*

Dispersion models estimate ambient levels of PM at a receptor site on the basis of emission factors for the relevant sources and the investigator's ability to model the advection, mixing, deposition, and chemical transformation of compounds from the source to the receptor site. Cass and Gray (1995), Gray and Cass (1998), and Kleeman and Cass (1998) have applied dispersion models to the South Coast Air Basin to estimate diesel PM concentrations. The models used by these investigators applied emission factors from 1982 and consequently are representative of concentrations prior to the implementation of diesel PM emission controls. In addition to offering another approach for estimating ambient diesel PM concentrations, the dispersion models summarized here provide the ability to distinguish on-highway from off-highway diesel source contributions and have presented an approach for quantifying the concentrations of secondary aerosols from diesel exhaust.

Cass and Gray (1995) used a Lagrangian particle-in-cell model to estimate the source contributions to atmospheric fine carbon particle concentrations in the Los Angeles area, including diesel emission factors from on-highway and off-highway sources. Their dispersion model indicates that for 1982, the annual average ambient concentrations of diesel PM ranged from 1.9 $\mu g/m^3$ in Azusa, CA, to 5.6 $\mu g/m^3$ in downtown Los Angeles (Table 2-15). The contribution of on-highway sources to diesel PM ranged from 63.3% in downtown Los Angeles to 89% in West Los Angeles. Of the on-highway diesel contribution, the model predicted that for southern California, HD trucks comprised the majority (85%) of the diesel PM inventory and overall they contributed 66% of the diesel PM in the ambient air. Off-road sources of diesel exhaust include pumping stations, construction sites, shipping docks, railroad yards, and heavy equipment repair facilities. Cass and Gray (1995) also report that wintertime peaks in diesel PM concentrations can reach 10 $\mu g/m^3$.

Kleeman and Cass (1998) developed a Lagrangian model that examines the size and chemical evolution of aerosols including gas-to-particle conversion processes during transport. This model was applied to one-well characterized episode in Claremont County, CA, on August

Table 2-15. Modeled diesel PM2.1 for South Coast Air Basin in 1982[a]

Location	On-highway diesel PM, $\mu g/m^3$	Total diesel PM, $\mu g/m^3$	% On-highway
Azusa	1.4	1.9	75
Pasadena	2.0	2.5	78
Anaheim	2.7	3.5	78
Long Beach	3.5	4.6	76
Downtown Los Angeles	3.5	5.6	63
Lennox	3.8	4.7	81
West Los Angeles	3.8	4.3	89

[a]Adapted from Cass and Gray (1995), modeling results of Gray (1986).

27-28, 1987. The model provided reasonable predictions of PM10 (overpredicting PM10 13%), elemental and organic carbon, and adequately reconstructed the size distribution of the aerosols. The model indicated that on August 27-28, 1987, the PM2.5 concentration was 76.7 $\mu g/m^3$, 13.2% of which (10.1 $\mu g/m^3$) was attributable to diesel engine emissions. This estimate includes secondary aerosol formation for sulfate, ammonium, nitrate, and organic compounds, which accounted for 4.9 $\mu g/m^3$ of the total estimated diesel PM mass. The secondary organic aerosol was estimated to be 1.1 $\mu g/m^3$, or 31% of the total seconary aerosol mass, with the remainder composed of nitrate, ammonium, and sulfate aerosols.

Dispersion modeling is also being conducted by EPA to estimate county-specific concentrations of, and exposures to, several toxic species, including diesel PM. Results from this model are expected in 2000.

2.4.3. Exposures to Diesel PM

Up to this point, the information on diesel PM has focused on estimates of concentrations in outdoor environments. Ultimately, it is personal exposure that determines health impacts. Personal exposures can be measured using surrogate chemical species such as EC, or exposures can be modeled. Results of both exposure assessment methods are discussed below.

Occupational exposures to diesel PM were reported for long-distance truck drivers, local drivers, mechanics, and dockworkers by Zaebst et al. (1991), and other occupational exposures are summarized by Watts (1995) and Birch and Cary (1996). Two modeling efforts have been developed to determine diesel PM exposures in the general population: the Hazardous Air

Pollutant Exposure Model-Mobile Sources version 3 (HAPEM-MS3) and the California Population Indoor Exposure Model (CPIEM).

2.4.3.1. *Exposure Measurements*

Occupational exposures to diesel PM were estimated by Zaebst et al. (1991), who reported EC measurements from personal samplers worn by road drivers, local drivers, dockworkers, and mechanics for 8-hour shifts at each of six large hub truck terminals. Residential background and highway background samples at fixed sites were also collected. Zaebst et al. (1991) reported warm- and cold-weather EC concentrations in residential background and highway background environments, which ranged from 0.9 $\mu g/m^3$ to 4.9 $\mu g/m^3$. Elemental carbon exposures for road and local truckers ranged from 2.0 $\mu g/m^3$ to 7.0 $\mu g/m^3$, while exposure levels for mechanics and dockworkers were reported between 4.8 $\mu g/m^3$ and 28.0 $\mu g/m^3$.

The geometric mean of the EC concentrations reported by Zaebst et al. (1991) was adjusted for the potential contribution of other EC sources using the ratiometric approach described above. The estimated diesel PM exposures calculated range from 3.5 $\mu g/m^3$ and 3.7 $\mu g/m^3$ for road and local drivers, respectively, to 12.6 $\mu g/m^3$ for mechanics (Table 2-16). Important variables in this calculation include the potential contribution of 2-stroke diesel engines (which generate PM with lower EC concentrations than 4-stroke diesel engines) and the contribution of other EC sources such as cigarette smoke, wood smoke, or gasoline combustion above the level accounted for. The exposure levels for road and local drivers to diesel PM estimated from the Zaebst et al. (1991) study are a factor of 2 to 3 higher than recent ambient PM levels reported for Denver, which is reasonable given that drivers are likely to be in closer proximity to traffic than at either of the two Denver fixed sites.

Additional occupational exposures to EC have been reported for miners, fire engine operators in engine houses, automotive repair shops dedicated to diesel vehicles, service bay workers in a public transit system, and aircraft ground crews (Birch and Cary, 1996; Watts, 1995). Diesel PM exposures were calculated from the EC exposures using an upper-bound estimate (e.g., EC concentration = diesel PM/0.7) because the workers were generally in confined spaces in which diesel exhaust was the dominant source of EC. If this upper-bound estimate is applied, the calculated occupational exposures to diesel PM range from 10 to 21 $\mu g/m^3$ for an aircraft ground crew, and up to 43 $\mu g/m^3$, 113 $\mu g/m^3$, and 140 $\mu g/m^3$ for bus public transit areas, firefighters in the station house, and bus transit service bay personnel, respectively. In additional occupational settings, breathing zone concentrations of EC have been reported by Birch and Cary

Table 2-16. Diesel PM1.0 exposures reported by Zaebst et al. (1991) and calculated using the EC ratiometric approach[a]

Location/job type	Number of samples	Geom. mean diesel PM (stdev) $\mu g/m^3$	Calculated diesel PM[a], $\mu g/m^3$
Residential background	23	1.1 (2.0)	1.0
Highway background	21	2.5 (2.4)	2.3
Road drivers	72	3.8 (2.3)	3.5
Local drivers	56	4.0 (2.0)	3.7
Dockworkers	75	12.1 (3.7)	11.1
Mechanics	80	13.8 (3.6)	12.6

[a] Diesel PM=(EC*0.64)/0.7.

(1996) and the estimated maximum diesel PM concentrations from these measurements suggest levels of 24 $\mu g/m^3$ for a beverage distributor warehouse, 100 $\mu g/m^3$ for diesel automotive repair crews, 286 $\mu g/m^3$ for a front end loader operator in a confined space of a timber processing plant, and 976 $\mu g/m^3$ for a firehouse bay area.

Watts (1995) reports diesel PM sampling conducted in mines during significant diesel activity, which does not represent personal exposures, but is a snapshot of short periods of elevated concentration that comprise a portion of a worker's daily exposure. The levels of diesel PM in four mines ranged from 850 $\mu g/m^3$ to 3260 $\mu g/m^3$. In a study of four railroads, Woskie et al. (1988) reported concentrations of respirable dust (corrected for cigarette particulate) that ranged from 17 $\mu g/m^3$ for clerks to 130 $\mu g/m^3$, 134 $\mu g/m^3$, and 191 $\mu g/m^3$ for supervisors, electricians, and hostlers, respectively. Although these exposures may have included nondiesel PM, the majority of the respirable PM is believed to have originated from the locomotive emissions.

2.4.3.2. *Modeling Exposures to Diesel PM*

Modeled estimates of individual exposures to diesel PM must integrate exposure in the various indoor and outdoor environments in which different individuals are active. Consequently, the demographic distribution, time-activity patterns, and diesel PM concentrations in the various environments, including job-related exposures, must all be taken into account.

2.4.3.2.1. ***The Hazardous Air Pollutant Exposure Model for Mobile Sources, version 3.*** The HAPEM-MS3 model is based on the carbon monoxide (CO) probabilistic NAAQS exposure model (CO pNEM), which is used to estimate the frequency distribution of population exposure to CO and the resulting carboxyhemaglobin levels (Law et al., 1997). The HAPEM model simulates the CO exposure scenario of individuals in 22 demographic groups for 37 microenvironments. CO concentrations are based on ambient measurements made in 1990 and are related to exposures of individuals in a 10 km radius around the sampling site. Diesel PM (DPM) exposures are calculated as in Equation 2-4, using a ratiometric approach to CO.

$$DPM_{ug/m^3} = (CO_{ug/m^3} / CO_{g/mi}) \times DPM_{g/mi} \qquad (2\text{-}4)$$

Input to the model includes CO monitoring data for 1990, time-activity data collected in Denver, CO, Washington DC, and Cincinnati, OH, from 1982 to 1985, microenvironmental data, and 1990 census population data. Motor vehicle diesel PM and CO emission rates reported by EPA (1999b) are used to calculate mobile-source diesel PM exposures. While gasoline vehicles emit the large majority of CO, gasoline and diesel highway vehicles travel on the same roadways, albeit with different spatial and temporal patterns. Nevertheless, the assumption can be made that the highway fleet (gasoline+diesel) emissions ratio of CO to diesel PM can be used as an adjustment factor to convert known or estimated CO personal exposure to diesel PM exposure estimates.

This modeling approach was first used to estimate population average exposures among 22 demographic groups for 9 urban areas (U.S. EPA, 1999b). The exposures were calculated based on air districts, which were defined as the population within the 10 km radius of the CO monitor. Employing average CO exposures in this approach may underestimate by approximately 30% the exposures experienced by the 98th percentile population (Law et al., 1997). In order to characterize exposures in these highly exposed populations, Brodowicz (1999) used CO concentrations relevant to the most highly exposed populations to determine diesel PM exposures for different demographic groups within this population. Results for both the annual average diesel PM exposures and exposures in the most highly exposed demographic groups are given in Table 2-17.

The annual average diesel PM exposures ranged from 0.6 μg/m^3 in Spokane, WA, to 1.7 μg/m^3 in New York (Table 2-17). The highest diesel PM exposures ranged from 0.9 μg/m^3 for outdoor workers in St. Louis to 4.1 μg/m^3 for outdoor children in New York (Table 2-17). The highest exposed demographic groups were those who spend a large portion of their time outdoors. Overall, the highest exposed individuals experienced diesel PM levels that were on

Table 2-17. Annual average diesel PM exposures for 1990 in the general population and among the highest exposed demographic groups in nine urban areas (on-road sources only)

Urban area	Population average exposure, $\mu g/m^3$	Highest diesel PM exposure, $\mu g/m^3$ (demographic group experiencing this exposure)
Chicago, IL	0.8	1.3 (outdoor workers)
Denver, CO	0.8	1.3 (outdoor workers)
Houston, TX	0.6	0.9 (outdoor workers)
Minneapolis, MN	1.0	1.6 (outdoor workers)
New York, NY	1.7	4.1 (outdoor children)
Philadelphia, PA	0.7	1.3 (outdoor children)
Phoenix, AZ	1.4	2.6 (nonworking men 18-44)
Spokane, WA	1.3	2.0 (outdoor workers)
St. Louis, MO	0.6	0.9 (outdoor workers)

average 1.7 times higher than the population average. Exposures to diesel PM in rural areas nationwide were estimated by HAPEM-MS3 to be 0.5 $\mu g/m^3$. It is important to note that these exposure estimates are lower than the total exposure to diesel PM because they reflect only diesel PM from on-road sources (U.S. EPA, 1998b).

Diesel PM exposure projections were calculated using the HAPEM model for 1996, 2007, and 2020 with a base case and a case assuming increased penetration of diesel engines in the LD truck fleet. The base case uses baseline fuels and emission rates, assuming the implementation of a National Low-Emission Vehicle (NLEV) program. The increased dieselization case uses baseline emission factors with Tier 2 standards and an assumed increase in LD diesel truck implementation equivalent to 50% of the LD truck sales beginning in model year 2004 (which is more aggressive than the likely increase in diesel engine market share).

Predicted diesel PM exposures for 2007 and 2020 decrease from 1996 levels by an average 55% and range from a low of 0.3 $\mu g/m^3$ in St. Louis to a high of 0.6 $\mu g/m^3$ in Phoenix, for both 2007 and 2020 (Table 2-18). The predicted decreases are a result of fleet turnover and the full implementation of Federal regulations that are currently in place. If the modeled increase

Table 2-18. Projected annual average diesel PM exposures from all on-road vehicles

Area	Diesel PM exposure by calendar year, μg/m³		
	1996	2007	2020
Chicago, IL	0.6	0.3	0.3
Denver, CO	0.7	0.4	0.4
Houston, TX	0.8	0.3	0.3
Minneapolis, MN	0.9	0.4	0.4
New York, NY	1.1	0.5	0.5
Philadelphia, PA	0.6	0.3	0.2
Phoenix, AZ	1.2	0.6	0.6
Spokane, WA	1.0	0.5	0.5
St. Louis, MO	0.5	0.3	0.2

in diesels in the LD truck fleet occurs, projected diesel PM exposures are expected to increase 38% on average over 1996 exposures. If diesel engines reached 50% of the light duty truck sales in 2010, instead of 2004, the increase in diesel PM exposure would be about 30%.

2.4.3.2.2. ***The California Population Indoor Exposure Model.*** The California Population Indoor Exposure Model (CPIEM), developed under contract to the California Air Resources Board, estimates Californians' exposure to diesel PM using distributions of input data and a Monte Carlo approach (California EPA, 1998a). This model uses population-weighted outdoor diesel PM concentrations in a mass-balance model to estimate diesel PM concentrations in four indoor environments: residences, office buildings, schools, and stores/retail buildings. The model takes into account air exchange rates, penetration factors, and a net loss factor for deposition/removal. In four additional environments (industrial plants, restaurants/lounges, other indoor places, and enclosed vehicles), assumptions were made about the similarity of each of these spaces to environments for which diesel PM exposures had been calculated. Industrial plants and enclosed vehicles were assumed to have diesel PM exposures similar to those in the outdoor environment, restaurant/lounges were assumed to have diesel PM concentrations similar to stores, and other indoor places were assumed to have diesel PM concentrations similar to offices. The estimated diesel PM concentrations in the indoor and outdoor environments range from 1.6 μg/m³ to 3.0 μg/m³ (Table 2-19).

Table 2-19. Modeled and estimated concentrations of diesel PM in microenvironments (California EPA, 1998a)

Environment	Estimated mean diesel PM (stdev), μg/m³
Residences	1.9 (0.9)
Offices	1.6 (0.7)
Schools	1.9 (0.8)
Stores/public/retail bldgs	2.1 (0.9)
Outdoor places	3.0 (1.1)
Industrial plants[a]	3.0 (1.1)
Restaurants/lounges[a]	2.1 (0.9)
Other indoor places[a]	1.6 (0.7)
Enclosed vehicles[a]	3.0 (1.1)

[a]Concentrations assumed based on similarity with modeled environments.

The diesel PM concentrations reported in Table 2-19 were used as input to the CPIEM model, and time-activity patterns for children and adults were used to estimate total indoor and total air exposures to diesel PM. Overall, total indoor exposures were estimated at 2.0 ± 0.7 μg/m³ and total air exposures (indoor and outdoor exposures) were 2.1 ± 0.7 μg/m³ (Table 2-20). The South Coast Air Basin and the San Francisco Bay Area were also modeled using CPIEM, where total air exposures to diesel PM were estimated to be 2.5 ± 0.9 μg/m³ and 1.7 ± 0.9 μg/m³, respectively.

Exposure estimates were also made by California EPA (1998a) for 1995, 2000, and 2010 using a ratiometric approach to 1990 exposures. Total air exposures reported for 1995 and projected for 2000 and 2010 were 1.5 μg/m³, 1.3 μg/m³, and 1.2 μg/m³, respectively.

2.4.4. Ambient Diesel PM Summary

It appears from the limited number of studies available that annually averaged diesel PM concentrations at fixed sites in urban and suburban areas in the 1980s ranged from approximately 4.4 μg/m³ to 11.6 μg/m³. CMB and dispersion modeling indicate that diesel PM concentrations on some winter days may reach 22 μg/m³ and on episode days concentrations of 10 μg/m³ are possible. CMB modeling results, which include emissions and measurements from 1990 and

Table 2-20. Estimated indoor air and total air exposures to diesel PM in California in 1990

Exposed population	Total indoor exposure (stdev), $\mu g/m^3$	Total air exposure, (stdev), $\mu g/m^3$
All Californians	2.0 (0.7)	2.1 (0.8)
South Coast Air Basin	2.4 (0.9)	2.5 (0.9)
San Francisco Bay Area	1.7 (0.9)	1.7 (0.9)

later, indicate that diesel PM concentrations in "hotspots" may reach 46.7 $\mu g/m^3$; diesel PM concentrations at fixed sites in urban and suburban areas range from approximately 1.2 $\mu g/m^3$ to 3.6 $\mu g/m^3$. Annual average diesel PM concentrations in rural and remote areas of the country are less than 1.0 $\mu g/m^3$.

Measurements of the diesel PM surrogate, EC, in rural and urban environments indicate concentrations similar to those reported by CMB methods, with diesel PM concentrations less than 1.0 $\mu g/m^3$ in rural areas and concentrations from approximately 0.5 $\mu g/m^3$ to 5.9 $\mu g/m^3$ in urban areas. The EC surrogate approach also provides some estimates of diesel PM in microenvironments such as in-vehicle concentrations (2.8-36.6 $\mu g/m^3$), near roadways with diesel traffic (0.7-7.5 $\mu g/m^3$ higher than background), and in schools (0.9-5.5 $\mu g/m^3$). Measurements of EC in occupational environments indicate that diesel PM exposures range from approximately 3.5 $\mu g/m^3$ for long distance diesel truck drivers to 140 $\mu g/m^3$ for bus transit service bay personnel.

It is noteworthy that the annually averaged concentrations and exposures will mask potentially important excursions experienced during episodic conditions, and/or seasonal elevations that may have important associated health risks. Individuals for whom exposures are equal to, or may greatly exceed, the annual average ambient diesel PM levels reported here may include those who spend a significant portion of their day on or near roadways, such as sales representatives, delivery personnel, construction workers, and individuals living in the vicinity of "hotspots" (near highway, bus depot, or other transit facility).

The HAPEM-MS3 exposure model, which assesses exposures from on-road diesel emissions only, indicates that on an annual basis the urban population is exposed to levels of diesel PM from 0.6 $\mu g/m^3$ to 1.7 $\mu g/m^3$. Diesel PM exposures for the most highly exposed individuals in urban areas are estimated by HAPEM-MS3 to range from 0.9 $\mu g/m^3$ to 4.1 $\mu g/m^3$,

with individuals spending a large amount of time outside comprising the highest exposure group. The California EPA (1998a) exposure model, which assesses diesel PM exposures from on- and off-road sources, reports diesel PM exposures for Californians ranging from 1.7 $\mu g/m^3$ to 2.5 $\mu g/m^3$. Projected diesel PM exposure levels are expected to decrease by 2007 in the absence of an increase in LD diesel truck implementation.

2.5. SUMMARY

Dieselization of the trucking industry first occurred in the 1930s, and reached 40%-50% of the market for Class 7 and 8 trucks (based on sales data) by early 1960. By the late 1980s, more than 95% of heavy trucks used diesel engines. Dieselization of locomotives began at the end of the Second World War and was completed rapidly, probably by the early 1950s. Technology innovations that impact emissions have occurred in the years since 1960, in particular the advent of turbocharging with charge air cooling, and DI engines. These advances have tended to lower emissions, but until the late 1980s engines were optimized for performance rather than emissions, so the effect was small. Overall, it is expected that engines in the 1950 to 1980s time frame would have PM emissions similar to those of the mid-1980 engines that were not yet controlled for particulates.

The proportion of 2-stroke engines in the in-use truck fleet was in all likelihood 20%-25% for most of the time from 1960 to 1985. Only in the late 1980s did 2-stroke engines begin to decline. Overall, regulated emissions changes due to changing proportions of 2- and 4-stroke engines in the in-use fleet during the years 1949-1975 do not appear to be significant for HD truck and bus engines. No significant difference in PM mass emissions between 2- and 4-stroke vehicles are evident; however, 2-stroke engines emitted PM with a higher organic and higher amounts of VOCs than did 4-stroke engines.

Regulated emissions of CO, HC, and PM have declined significantly for on-road trucks since the mid-1970s. PM emissions appear to have decreased by a factor of 6 while emissions of NO_x have remained approximately constant. Emissions trends for earlier years are unknown; however, given that there were no emissions regulations in effect until the early 1970s it is likely that emissions were fairly constant during the 1960s. Little change in locomotive emissions from the early 1970s to the 1990s is evident. It is likely that this trend can also be extrapolated back to the mid-1950s.

Data on nonregulated emissions and particle size were reviewed. It is apparent that the soluble organic fraction of particulate, as well as the solid portion, have declined during the past two decades. EC content comprises the largest fraction of diesel PM and so has declined. There is also evidence for a decrease in the percentage of organics adsorbed on the particulates over

time. Emissions of PAHs and nitro-PAHs appear to have declined in parallel with emissions of total PM and SOF. There is no evidence to suggest that toxicologically significant organic components of DE (e.g., PAHs and nitroaromatics) have changed out of proportion to the change in organic mass.

Particle size measurements suggest that the size distribution for current emissions may be shifted more toward slightly higher number concentrations of nuclei-mode particles. However, methodologies for assessing nuclei particles are in a very early stage of technical development and no conclusions can be made at the current time.

The dilution of exhaust under roadway conditions is not well simulated by dynamometer dilution tunnel tests (dilution ratios of approximately 1000 in the ambient environment, compared to 10-fold dilution in laboratory tests). This discrepancy may lead to particle size distribution and gas-particle phase distributions of semivolatile compounds under conditions slightly different from those predicted from laboratory data. Diesel engines emit several toxicologically important compounds, including nitroarenes and other PAH compounds. The chemical and physical changes of diesel exhaust in the atmosphere have been extensively explored, but knowledge concerning the products of these chemical transformations is still limited and is challenging to predict from laboratory tests. In general, diesel exhaust components will become more oxidized in the atmosphere, making them more polar and therefore more water-soluble. Secondary aerosols from diesel exhaust may be removed at rates different from their precursor compounds, and may exhibit different biological reactivities.

Diesel PM concentrations reported from chemical mass balance studies in the 1980s suggest that annually averaged concentrations ranged from approximately 4.4 $\mu g/m^3$ to 11.6 $\mu g/m^3$. More recent analysis suggests that annually averaged ambient concentrations of diesel PM range from 0.2 $\mu g/m^3$ to 3.6 $\mu g/m^3$, with levels below 1.0 $\mu g/m^3$ for the more rural/remote areas. Chemical mass balance modeling and dispersion analysis suggest that in urban hot spots and during episodic conditions, diesel PM concentrations may be as high as 10-47 $\mu g/m^3$. Exposure modeling has indicated that individuals from the general population in urban areas may be exposed to 0.6-1.7 $\mu g/m^3$ diesel PM, while those individuals who spend a large amount of their time out of doors may have exposures ranging up to 4.1 $\mu g/m^3$. Diesel PM exposures in some occupational environments can exceed these levels by 2-3 orders of magnitude.

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3. DOSIMETRY OF DIESEL EXHAUST PARTICLES IN THE RESPIRATORY TRACT

3.1. INTRODUCTION

This chapter presents the data and current scientific thought on the deposition and clearance of diesel particulate matter (DPM) from biological systems as well as discussion of the measures of DPM in tissues. The overall goal of the chapter is to address the issue of animal-to-human extrapolation by integrating these data and thoughts into an estimate of a "human equivalent concentration" (HEC), i.e., the concentration in humans corresponding to those used in animal studies. Models that codify and integrate these data and thoughts to estimate the HEC are described and evaluated. This information is needed to inform the dose-response and extrapolation analyses in Chapter 6 and to facilitate understanding of animal carcinogenicity and the bioavailability of particle organics in the lung.

The major constituents of diesel engine exhaust and their atmospheric reaction products are described in Chapter 2 and in the report on diesel exhaust issued by the Health Effects Institute (Health Effects Institute, 1995). Diesel engine exhaust consists of a complex mixture of typical combustion gases, vapors, low-molecular-weight hydrocarbons, and particles; it is the particle phase that is of greatest health concern.

Because pulmonary toxicity is the major focal point, dosimetric considerations are limited to the lung. The dosimetric aspects of DPM to be considered in this chapter include the characteristics of DPM, deposition of DPM in the conducting airways and alveolar regions, normal DPM clearance mechanisms and rates of clearance in both regions, clearance rates during lung overload, elution of organics from DPM, transport of DPM to extra-alveolar sites, and the interrelationships of these factors in determining the target organ dose. 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 and the relevance of the tumorigenic response in rats to humans questionable.

3.2. CHARACTERISTICS OF INHALED DPM AND RELATIONSHIP TO $PM_{2.5}$

The formation, transport, and characteristics of DPM are considered in detail in Chapter 2 and in the report on diesel exhaust (Health Effects Institute, 1995). DPM consists of aggregates of spherical carbonaceous particles (about 0.2 μm MMAD) to which significant amounts of higher-molecular-weight organic compounds are adsorbed (Figure 2-1) as the hot engine exhaust is cooled to ambient temperature. DPM has an extremely large surface area that allows for the

adsorption of organic compounds. Typically, 10% to 40% of DPM mass consists of organic compounds (Health Effects Institute, 1995). This figure compares with mass apportionment of 20.9% to organic compounds in $PM_{2.5}$ samples collected at sites in the eastern United States (U.S. EPA, 1996). These organic chemicals include high-molecular-weight hydrocarbons such as the polyaromatic hydrocarbons (PAHs) and their derivatives. DPM also contains a sulfate component that varies with the sulfur content of the fuel. DPM in areas such as Los Angeles and Denver makes up about 7% and 10%, respectively, of the fine particulate matter (PM) fraction (Health Effects Institute, 1995; Zielinska et al., 1998). In another study of fine particulate mass concentration in southern California, the percentage apportioned to diesel exhaust was even higher, ranging from 33% in downtown Los Angeles to 14% in a suburban/rural area in California (Schauer et al., 1996).

3.3. REGIONAL DEPOSITION OF INHALED DPM

This section discusses the major factors controlling the disposition of inhaled particles. Note that disposition is defined as encompassing the processes of deposition, absorption, distribution, metabolism, and elimination. 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, density, and electrostatic attraction) 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 DPM 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, and only a brief review will be presented here. 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; U.S. EPA, 1996).

The respiratory tract in both humans and experimental mammals can be divided into three regions on the basis of structure, size, and function (International Commission on Radiological Protection, 1994): the extrathoracic (ET), the tracheobronchial (TB), and the alveolar (A). In humans, inhalation can occur through the nose or mouth or both (oronasal breathing). However, many animal models used in respiratory toxicology studies are obligate nose breathers.

3.3.1. Deposition Mechanisms

This section provides an overview of the basic mechanisms by which inhaled particles deposit within the respiratory tract. Details concerning the aerosol physics that explain both how and why particle deposition occurs as well as data on total human respiratory tract deposition are presented in detail in the earlier PM Criteria Document (U.S. EPA, 1996) and will only be briefly reviewed here. For more extensive discussions of deposition processes, refer to reviews by Morrow (1966), Raabe (1982), U.S. EPA (1982), Phalen and Oldham (1983), Lippmann and Schlesinger (1984), Raabe et al. (1988), and Stöber et al. (1993).

Particles may deposit by five major mechanisms (inertial impaction, gravitational settling, Brownian diffusion, electrostatic attraction, and interception). The relative contribution of each deposition mechanism to the fraction of inhaled particles deposited varies for each region of the respiratory tract.

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 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 extrathoracic (or nasopharyngeal), tracheobronchial, and alveolar regions of the respiratory tract.

Upon inhalation of particulate matter such as found in diesel exhaust, deposition will occur throughout the respiratory tract. Because of high airflow velocities and abrupt directional changes in the ET and TB regions, inertial impaction is a primary deposition mechanism, especially for particles ≥ 2.5 μm d_{ae} (aerodynamic equivalent diameter). 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, which have an $d_{ae} \leq 1$ μm and a small aspect ratio (ratio of the length to diameter).

All aerosol particles are continuously influenced by gravity, but particles with a $d_{ae} > 0.5$ μm are affected to the greatest extent. A spherical, compact particle will acquire a terminal settling velocity when a balance is achieved between the acceleration of gravity acting on the particle and the viscous resistance of the air; it is this velocity that brings the particle into contact with airway surfaces. Both sedimentation and inertial impaction cause the deposition of many particles within the same size range. These deposition processes act together in the ET and TB regions, with inertial impaction dominating in the upper airways and sedimentation becoming increasingly dominant in the lower conducting airways, especially for the largest particles, which can penetrate into the smaller bronchial airways.

As particle diameters become <1 μm, the particles are increasingly subjected to diffusive deposition because of random bombardment by air molecules, which results in contact with airway surfaces. A d_{ae} of 0.5 μm is often considered as a boundary between diffusion and

aerodynamic (sedimentation and impaction) mechanisms of deposition. Thus, instead of having an aerodynamic equivalent diameter (d_{ae}), diffusive particles of different shapes can be related to the diffusivity of a thermodynamic equivalent size based on spherical particles (Heyder et al., 1986). Diffusive deposition of particles is favored in the A region of the respiratory tract by the proximate surfaces and by relatively long residence times for particles.

Because their d_{ae} is generally $\leq$ 1 μm, diesel exhaust particles may deposit throughout the respiratory tract. On the basis of 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 DPM and/or the adsorbed organics. However, such data for humans are not available. As discussed above, deposition by diffusion would be especially prevalent in the A region, whereas sedimentation would be less significant for such small particles.

Electrostatic precipitation is deposition related to particle charge. The electrical charge on some particles may result in an enhanced deposition over what would be expected from size alone. This is due to image charges induced on the surface of the airway by these particles, or to space-charge effects whereby repulsion of particles containing like charges results in increased migration toward the airway wall. The effect of charge on deposition is inversely proportional to particle size and airflow rate. A recent study employing hollow airway casts of the human tracheobronchial tree that assessed deposition of ultrafine (0.02 μm) and fine (0.125 μm) particles found that deposition of singly charged particles was 5-6 times that of particles having no charge, and 2-3 times that of particles at Boltzmann equilibrium (Cohen et al., 1998). This suggests that within the TB region of humans, electrostatic precipitation may be a significant deposition mechanism for ultrafine and some fine particles, the latter of which are inclusive of DPM. Thus, although electrostatic precipitation is generally a minor contributor to overall particle deposition, it may be important for DPM.

Interception is deposition by physical contact with airway surfaces and is most important for fiber deposition; interception is described in the 1996 CD.

3.3.1.1. *Biological Factors Modifying Deposition*

The available experimental deposition data in humans are commonly derived using healthy adult Caucasian males. Various factors can act to alter deposition patterns from those obtained in this group. The effects of different biological factors, including gender, age, and respiratory tract disease, on particle deposition have been reviewed previously (U.S. EPA, 1996).

The various species that serve as the basis for dose-response assessment in inhalation toxicology studies do not receive identical doses in a comparable respiratory tract region (ET, TB, or A) when exposed to the same aerosol or gas (Brain and Mensah, 1983). Such interspecies differences are important because the adverse toxic effect is likely more related to the

quantitative pattern of deposition within the respiratory tract than to the exposure concentration; this pattern determines not only the initial respiratory tract tissue dose but also the specific pathways by which the inhaled material is cleared and redistributed (Schlesinger, 1985). Differences in patterns of deposition between humans and animals have been summarized (U.S. EPA, 1996; Schlesinger et al., 1997). Such differences in initial deposition must be considered when relating biological responses obtained in laboratory animal studies to effects in humans.

The deposition of inhaled diesel particles in the respiratory tract of humans and mammalian species has been reviewed (Health Effects Institute, 1995). Schlesinger (1985) showed that physiological differences in the breathing mode for humans (nasal or oronasal breathers) and laboratory rats (obligatory nose breathers), combined with different airway geometries, resulted in significant differences in lower respiratory tract deposition for larger particles (>1 μm d_{ae}). 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 DPM in rats, hamsters, and humans on the basis of calculations of the models of Schum and Yeh (1980) and Weibel (1963). These simulations (Figure 3-1) indicate relative deposition patterns in the lower respiratory tract (trachea = generation 1; alveoli = generation 23) and are similar among hamsters, rats, and humans. Variations in alveolar deposition of DPM 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 3-1 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 3-1, the deposited dose, expressed as either mass/lung volume or mass/surface area(s), 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 deposited dose in relevant target areas are important and have to be considered

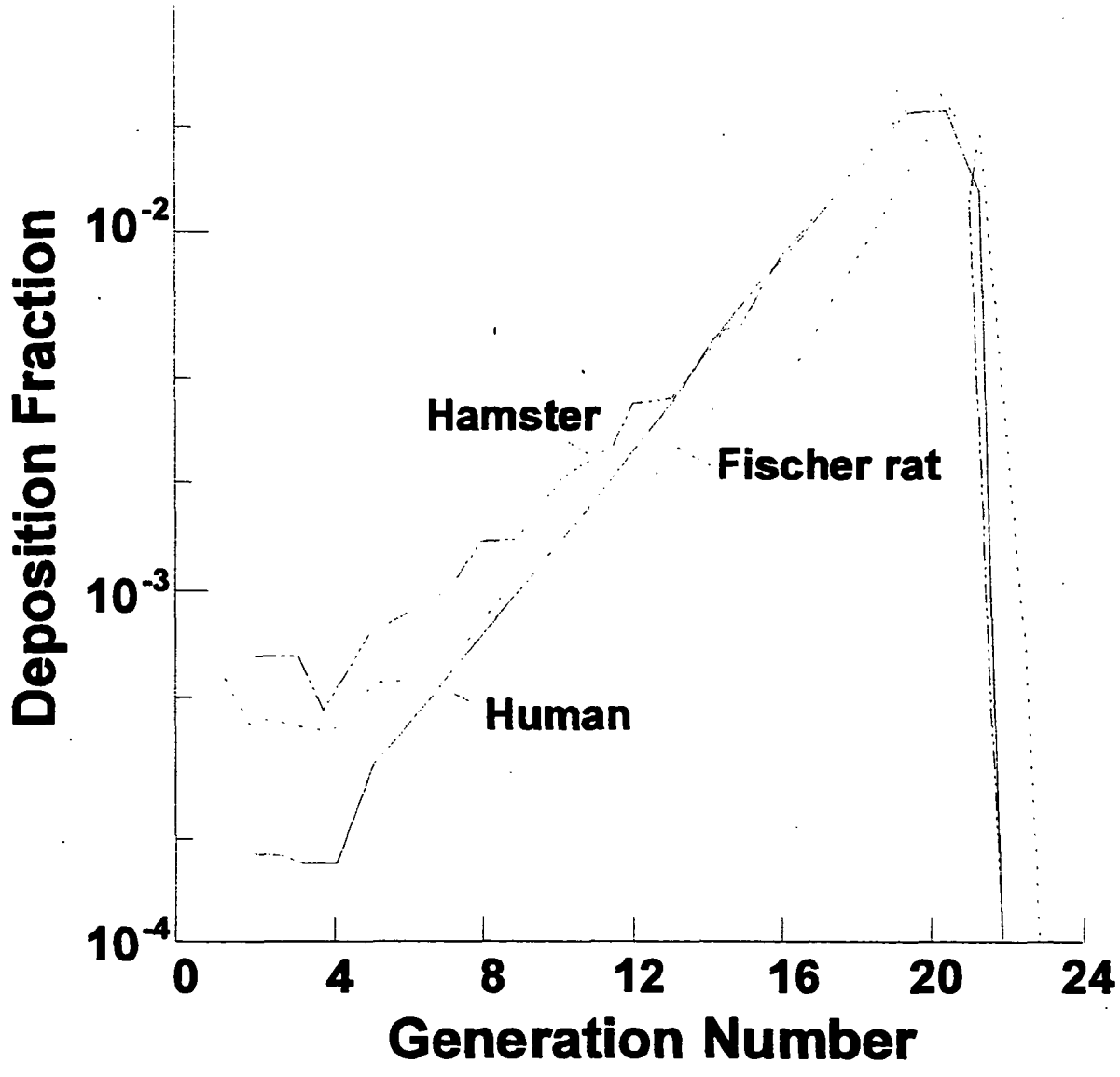

Figure 3-1. Modeled deposition distribution patterns of inhaled diesel exhaust particles in the airways of different species. Generation 1-18 are TB; >18 are A.

Source: Xu and Yu, 1987.

whenextrapolating the results from diesel exhaust exposure studies in animals to humans. Table 3-1 indicates that the differences (between humans to animals) are less on a surface area basis (≈3-fold) than on a lung volume basis (≈14-fold). This is due to larger alveolar diameters and concomitant lower surface area per unit of lung volume in humans.

Table 3-1. 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/min/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

$M = \frac{\text{mass DPM deposited in lung per minute}}{\text{total lung volume}}$

$M_1 = \frac{\text{mass DPM deposited in lung per minute}}{\text{total airway surface area}}$

$M_2 = \frac{\text{mass DPM deposited on the unciliated airways per minute}}{\text{surface area of the unciliated airways}}$

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.

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.

3.3.2. Particle Clearance and Translocation Mechanisms

This section provides an overview of the mechanisms and pathways by which particles are cleared from the respiratory tract. The mechanisms of particle clearance as well as clearance routes from the various regions of the respiratory tract have been considered in the previous PM Criteria Document (U.S. EPA, 1996) and reviewed by Schlesinger et al. (1997).

Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely, or may be translocated to other sites within this system, by various regionally distinct processes. These clearance mechanisms can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles) and may occur simultaneously or with temporal variations. Particle solubility in terms of clearance refers to solubility within the respiratory tract fluids and cells. Thus, an "insoluble" particle is one whose rate of clearance by dissolution is insignificant compared to its rate of clearance as an intact particle (as is the case

with DPM). For the most part, all deposited particles are subject to clearance by the same mechanisms, with their ultimate fate a function of deposition site, physicochemical properties (including any toxicity), and sometimes deposited mass or number concentration.

3.3.2.1. *ET Region*

The clearance of insoluble particles deposited in the nonolfactory portion of nasal passages occurs via mucociliary transport, and the general flow of mucus is backwards, i.e., towards the nasopharynx. Mucus flow in the most anterior portion of the nasal passages is forward, clearing deposited particles to the vestibular region where removal is by sneezing, wiping, or blowing.

Soluble material deposited on the nasal epithelium is accessible to underlying cells via diffusion through the mucus. Dissolved substances may be subsequently translocated into the bloodstream. The nasal passages have a rich vasculature, and uptake into the blood from this region may occur rapidly.

Clearance of poorly soluble particles deposited in the oral passages is by coughing and expectoration or by swallowing into the gastrointestinal tract.

3.3.2.2. *TB Region*

The dynamic relationship between deposition and clearance is responsible for determining lung burden at any point in time. Clearance of poorly soluble 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, as compared to alveolar clearance, which is on the order of 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 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 at the site of deposition. 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 3-2.

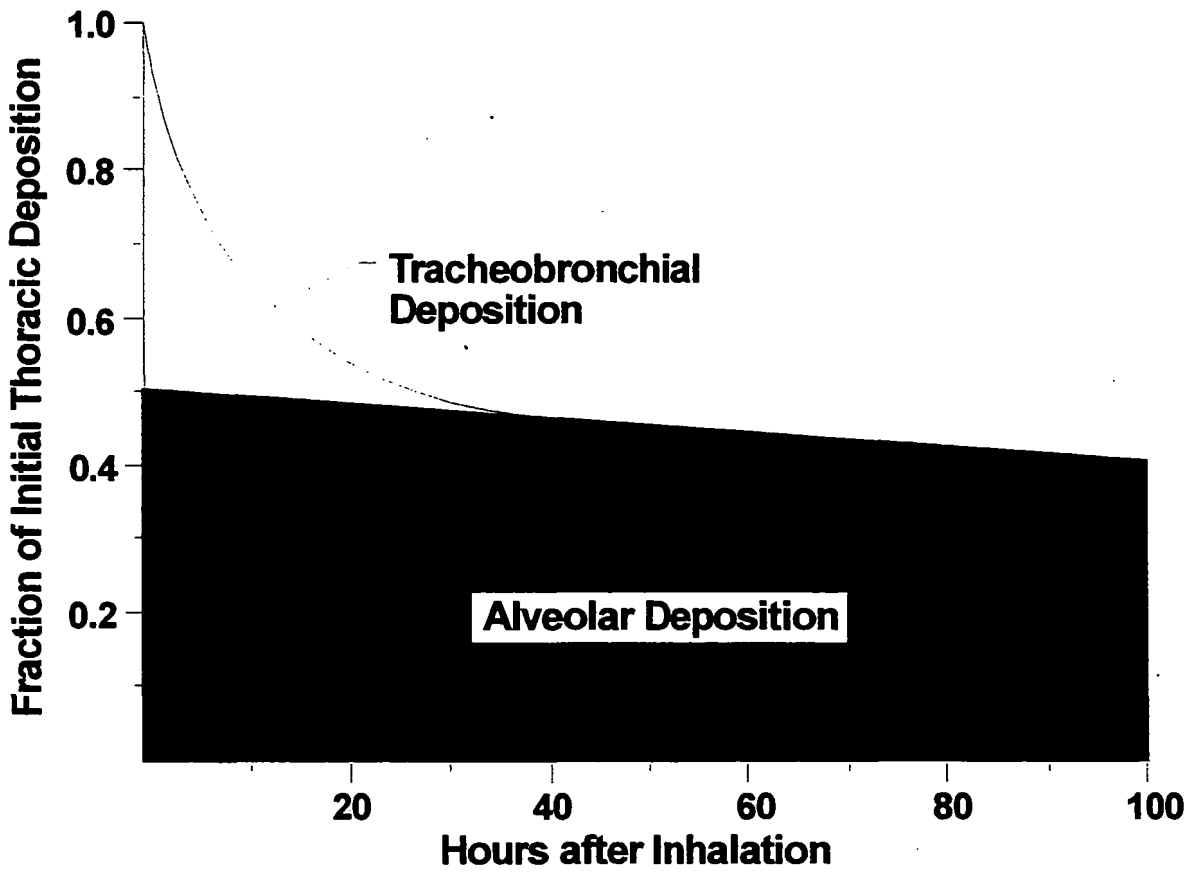

Figure 3-2. Modeled clearance of insoluble 4-μm particles deposited in tracheobronchial and alveolar regions in humans.

Source: Cuddihy and Yeh, 1986.

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: these include respiratory tract airway dimensions, calculated inhaled particle deposition fractions for individual airways, and thoracic (ALV + TB) 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 those for intratracheally instilled particles. The velocities observed for inhalation studies were generally lower than those of instillation studies. Figure 3-3 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 ^{99m}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 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 exposed to DPM. 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

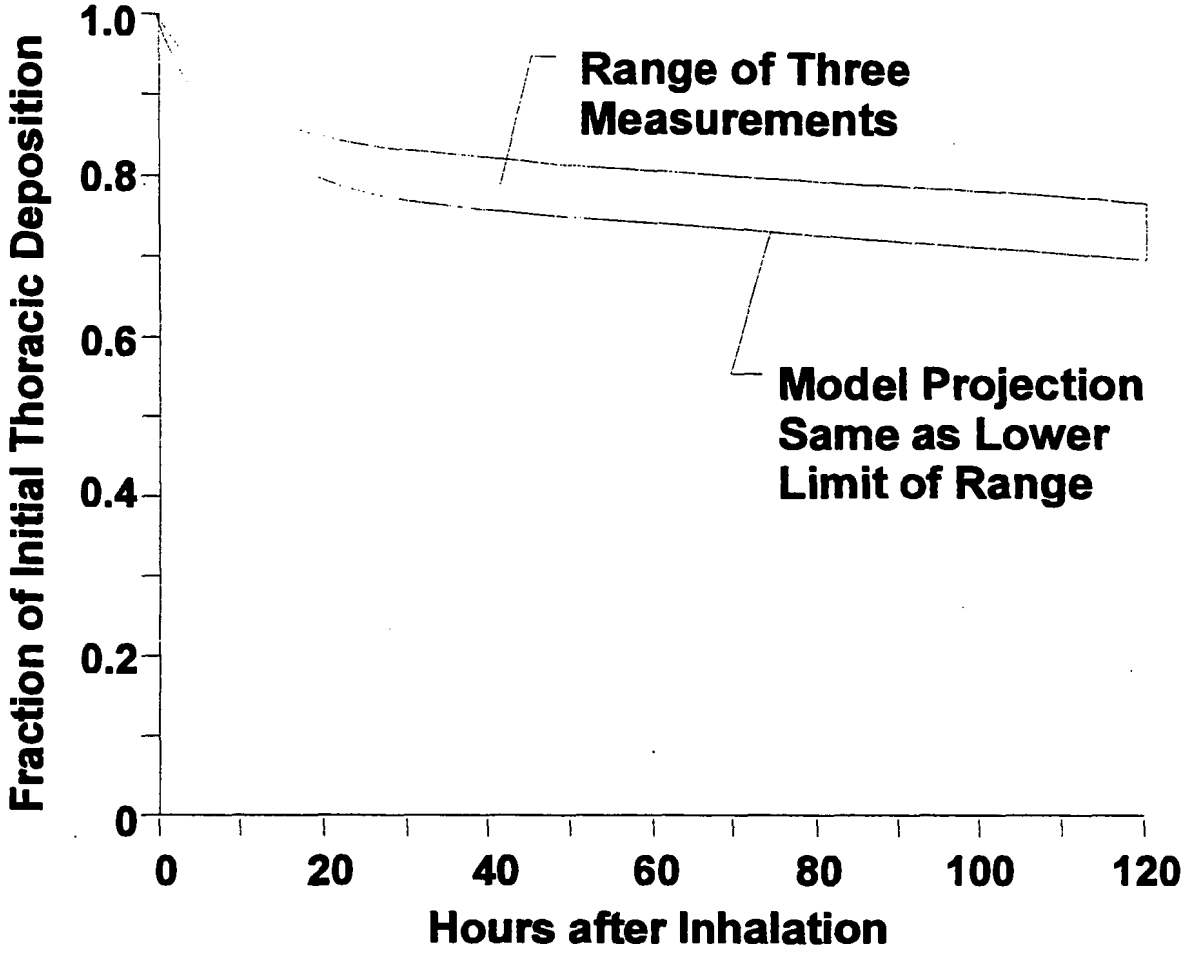

Figure 3-3. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

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

that release of polycyclic aromatic hydrocarbons (PAHs) from particles may occur within minutes and at the site of initial deposition.

The absence of effects in the TB areas in long-term DPM studies and experimental evidence that particle-associated PAHs are released at the site of particle deposition together suggest that these PAHs may be of lesser importance in tumorigenic responses of rats than originally suspected. However, as noted in Section 3.6, a larger fraction of particles are deposited in the interstitium of small airways in primates than in rats (Nikula et al., 1997). Moreover, eluted PAHs are retained longer than those in the alveoli (Gerde et al., 1999), allowing time for activation. Thus PAHs may play a greater role in humans exposed to DE.

Moreover, impairment of mucociliary clearance function as a result of exposure to occupational or environmental respiratory tract toxicants or to cigarette smoke may 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 present in diesel exhaust-induced rat pulmonary tumors.

Poorly soluble particles (i.e., DPM) deposited within the TB region are cleared predominantly by mucociliary transport, towards the oropharynx, followed by swallowing. Poorly soluble particles may also be cleared by traversing the epithelium by endocytotic processes, and enter the peribronchial region. Clearance may occur following phagocytosis by airway macrophages, located on or beneath the mucous lining throughout the bronchial tree, or via macrophages which enter the airway lumen from the bronchial or bronchiolar mucosa (Robertson, 1980).

3.3.2.3. *A Region*

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 of MMAD 1.9 and 6.1 μm (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 coal miners who had mild pneumoconiosis with presumably high lung burdens of coal dust. 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.

Normal alveolar clearance rates in laboratory animals exposed to DPM have been reported by a number of investigators (Table 3-2). 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 ($t_{1/2}$ of 1 day) elimination by mucociliary transport and a slower ($t_{1/2}$ 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 ($t_{1/2}$) 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 DPM/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,

Table 3-2. Alveolar clearance in laboratory animals exposed to DPM

Species/sex	Exposure technique	Exposure duration	Particles mg/m³	Observed effects	Reference
Rats, F-344, M	Nose only; Radiolabeled DPM	40-45 min	6	Four days after exposure, 40% of DPM eliminated by mucociliary clearance. Clearance from lower RT was in 2 phases. Rapid mucociliary ($t_{1/2}$ = 1 day; slower macrophage-mediated ($t_{1/2}$ = 62 days).	Chan et al. (1981)
Rats, F-344	Whole body; assessed effect on clearance of $^{67}Ga_2O_3$ particles	7 h/day 5 days/week 24 mo	0.35 3.5 7.0	τ_1 significantly higher with exposure to 7.0 mg/m³ for 24 mo; τ_2 significantly longer after exposure to 7.0 mg/m³ for 6 mo. and to 3.5 mg/m³ for 18 mo.	Wolff et al. (1986, 1987)
Rats	Whole body	19 h/day 5 days/week 2.5 years	4	Estimated alveolar deposition = 60 mg; particle burden caused lung overload. Estimated 6-15 mg particle-bound organics deposited.	Heinrich et al. (1986)
Rats, F-344, MF	Whole body	7 h/day 5 days/week 18 mo	0.15 0.94 4.1	Long-term clearance was 87 ± 28 and 99 ± 8 days for 0.15 and 0.94 mg/m³ groups, respectively; $t_{1/2}$ = 165 days for 4.1 mg/m³ group.	Griffis et al. (1983)
Rats, F-344;	Nose-only; Radiolabeled ^{14}C	45 min 140 min	7 2	Rats demonstrated 3 phases of clearance with $t_{1/2}$ = 1, 6, and 80 days, representing tracheobronchial, respiratory bronchioles, and alveolar clearance, respectively. Guinea pigs demonstrated negligible alveolar clearance from day 10 to 432.	Lee et al. (1983)
Guinea pigs, Hartley		45 min	7		
Rats, F-344		20 h/day 7 days/week 7-112 days	0.25 6	Monitored rats for a year. Proposed two clearance models. Clearance depends on initial particle burden; $t_{1/2}$ increases with higher exposure. Increases in $t_{1/2}$ indicate increasing impairment of AM mobility and transition into overload condition.	Chan et al. (1984)

RT = respiratory tract.
AM = alveolar macrophage.
τ_1 = clearance from primary, ciliated airways.
τ_2 = clearance from non-ciliated passages.

representing the early clearance from nonciliated passages such as alveolar ducts and alveoli, were noted after as few as 6 mo exposure to DPM at 7.0 mg/m^3 and 18 mo 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-mo 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, 6-15 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. Lung burdens were 0.035, 0.220, and 1.890 mg/g lung tissue, respectively, in rats exposed to 0.15, 0.94, and 4.1 mg DPM/m^3. 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 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 because the 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 much longer (>8 mo) than the average retention $t_{1/2}$ of 60 days for rats.

Clearance from the A region occurs via a number of mechanisms and pathways, but the relative importance of each is not always certain and may vary between species. Particle removal by macrophages comprises the main nonabsorptive clearance process in this region. Alveolar macrophages reside on the epithelium, where they phagocytize and transport deposited material, which they contact by random motion or via directed migration under the influence of local chemotactic factors (Warheit et al., 1988).

Particle-laden macrophages may be cleared from the A region along a number of pathways described in the 1996 CD. Uningested particles or macrophages in the interstitium may traverse the alveolar-capillary endothelium, directly entering the blood (Raabe, 1982; Holt, 1981); endocytosis by endothelial cells followed by exocytosis into the vessel lumen seems, however, to be restricted to particles <0.1 μm diameter, and may increase with increasing lung burden (Lee et al., 1985; Oberdörster, 1988). Once in the systemic circulation, transmigrated macrophages, as well as uningested particles, can travel to extrapulmonary organs.

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 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, numerous lines of evidence indicate that such AM-mediated particle clearance is 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 alveolar region of the lung 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 (1988, 1989) performed studies 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 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. 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 the particles may not exclusively reflect the translocation of AMs from the lung. The observations are also consistent with a gradual redistribution of retained particles among the AMs in the lung concurrent with the removal of particle-containing AMs via the conducting airways. Experimental support suggestive of potential processes for such particle redistribution comes from a variety of investigations involving AMs and other endocytic cells (Heppleston and Young, 1973; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978).

3.3.3. Translocations of Particles to Extra-alveolar 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 alveolar lining (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 engulfment (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 neutrophils (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 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 may have on the ability of the interstitial macrophage 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).

3.3.3.1. *Clearance Kinetics*

The clearance kinetics of PM have been reviewed in the PM CD (U.S. EPA, 1996) and by Schlesinger et al. (1997). Deposited particles may be completely or incompletely cleared from the respiratory tract. However, the time frame over which clearance occurs affects the cumulative dose delivered to the respiratory tract, as well as to extrapulmonary organs.

3.3.3.2. *Interspecies Patterns of Clearance*

The inability to study the retention of certain materials in humans for direct risk assessment requires the use of laboratory animals. Since dosimetry depends on clearance rates

and routes, adequate toxicological assessment necessitates that clearance kinetics in these animals be related to those in humans. The basic mechanisms and overall patterns of clearance from the respiratory tract are similar in humans and most other mammals. However, regional clearance rates can show substantial variation between species, even for similar particles deposited under comparable exposure conditions. This has been extensively reviewed in the previous document (U.S. EPA, 1996) and in other papers (Schlesinger et al., 1997; Snipes et al., 1989).

In general, there are species-dependent rate constants for various clearance pathways. Differences in regional and total clearance rates between some species are a reflection of differences in mechanical clearance processes. For consideration in assessing particle dosimetry, the end result of interspecies differences in clearance is that the retention of deposited particles can differ between species, which may result in differences in response to similar particulate exposure atmospheres.

3.3.3.3. *Biological Factors Modifying Clearance*

A number of host and environmental factors may modify normal clearance patterns. These include age, gender, physical activity, respiratory tract disease, and irritant inhalation (U.S. EPA, 1996).

3.3.3.4. *Respiratory Tract Disease*

Earlier studies reviewed in the PM CD (U.S. EPA, 1996) noted that various respiratory tract diseases are associated with clearance alterations. The examination of clearance in individuals with lung disease requires careful interpretation of results, since differences in deposition of tracer particles used to assess clearance function may occur between normal individuals and those with respiratory disease, and this would directly impact upon the measured clearance rates, especially in the tracheobronchial tree. Prolonged nasal mucociliary clearance in humans is associated with chronic sinusitis, bronchiectasis or rhinitis, and cystic fibrosis. Bronchial mucus transport may be impaired in people with bronchial carcinoma, chronic bronchitis, asthma, and various acute infections. In certain of these cases, coughing may enhance mucus clearance, but it generally is effective only if excess secretions are present.

The rates of A region particle clearance were reduced in humans with chronic obstructive lung disease and in laboratory animals with viral infections, while the viability and functional activity of macrophages were impaired in human asthmatics and in animals with viral-induced lung infections (U.S. EPA, 1996). However, any modification of functional properties of macrophages appears to be injury specific, reflecting the nature and anatomic pattern of disease.

3.4. PARTICLE OVERLOAD

3.4.1. Introduction

Some experimental studies using laboratory rodents employed high exposure concentrations of relatively nontoxic, poorly soluble particles. These particle loads interfered with normal clearance mechanisms, producing clearance rates different from those that would occur at lower exposure levels. Prolonged exposure to high particle concentrations is associated with what is termed particle overload. This is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway.

Wolff et al. (1987) used ^{134}Cs-labeled fused aluminosilicate particles to measure alveolar clearance in rats following 24-mo exposure to low (L), medium (M), and high (H) concentrations of diesel exhaust (targeted concentrations of DPM of 0.35, 3.5 and 7.0 mg/m^3). The short-term component of the multicomponent clearance curves was similar for all groups, but long-term clearance was retarded in the M and H exposure groups (Figure 3-4). The half times of the long-term clearance curves were 79, 81, 264, and 240 days, respectively, for the control, L, M, and The observed lung burdens increased progressively, reaching levels of 11.5 and 20.5 mg

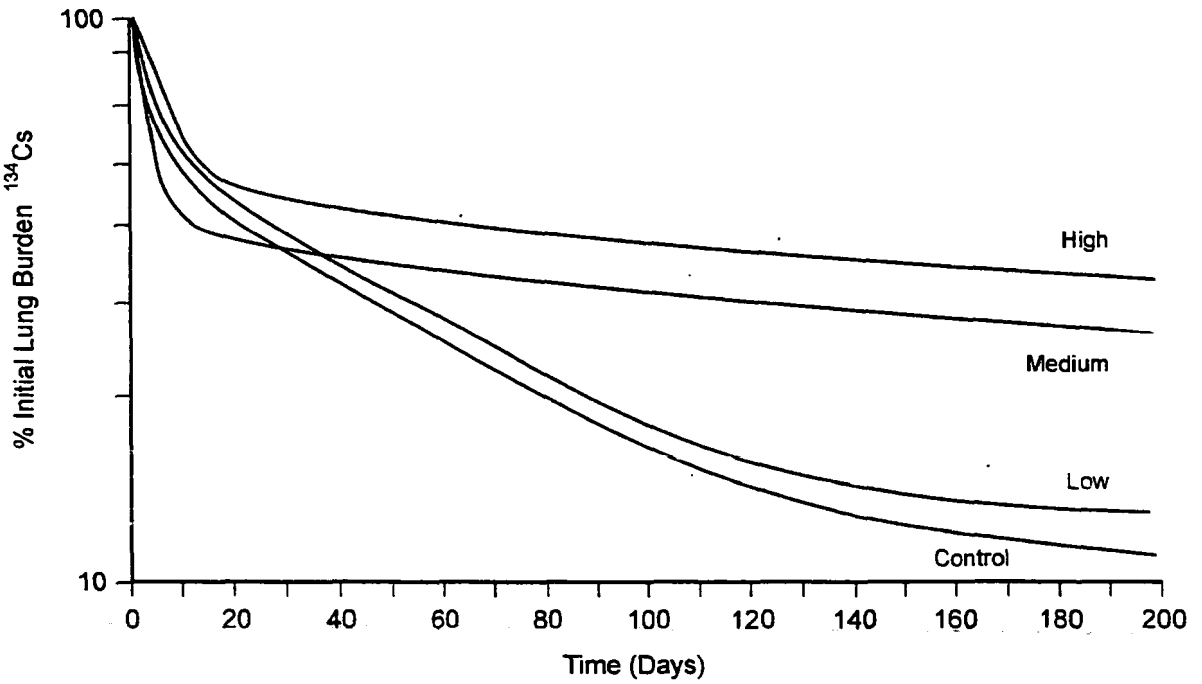

Figure 3-4. Clearance from lungs of rats of ^{134}Cs-FAP fused aluminosilicate tracer particles inhaled after 24 months of diesel exhaust exposure at concentrations of 0 (control) (●), 0.35 (low) (■), 3.5 (medium) (●), and 7.0 (high) mg DPM/m^3 (▲). Points on curves are means ± SE.

Source: Wolff et al., 1987.

H exposure groups. Clearance was overloaded at M and H exposure levels, but not by the L exposure level. Lung burdens of DPM were measured after 6, 12, 18, and 24 mo of exposure. DPM/lung, respectively, after 24 mo in the M and H exposed groups (Figure 3-5). The results indicate that the clearance of freshly deposited particles was retarded after 24 mo of DPM exposure at the two highest exposure levels, and that clearance had become overloaded at these two exposures but not at the lowest exposure.

It has been hypothesized that overloading will begin in the rat when deposition approaches 1 mg particles/g lung tissue (Morrow, 1988). When the concentration reaches 10 mg particles/g lung tissue, macrophage-mediated clearance of particles would effectively cease. It is a nonspecific effect noted in experimental studies, generally in rats, using many different kinds of poorly soluble particles (including TiO_2, volcanic ash, DPM, carbon black, and fly ash) and results in A region clearance slowing or stasis, with an associated inflammation and aggregation of macrophages in the lungs and increased translocation of particles into the interstitium (Muhle et al., 1990; Lehnert, 1990; Morrow, 1994). Following overloading, the subsequent retardation

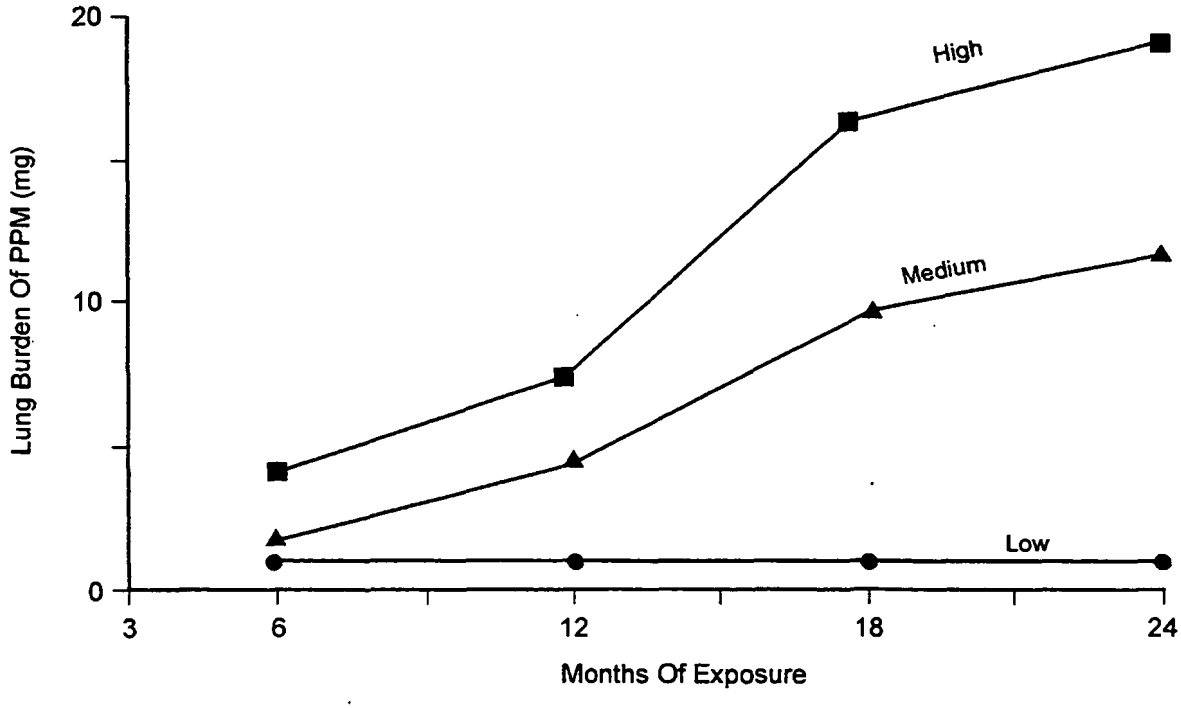

Figure 3-5. Lung burdens of DPM within rats exposed to 0.35 (low) (●), 3.5 (medium) (▲), and 7.0 (high) mg ppm/m^3 (■).

Source: Wolff et al., 1987.

of lung clearance, accumulation of particles, inflammation, and the interaction of inflammatory mediators with cell proliferative processes and DNA may lead to the development of tumors and fibrosis in rats (Mauderly, 1996). The phenomenon of overload has been discussed in greater detail in the previous PM CD (U.S. EPA, 1996).

3.4.2. Relevance to Humans

The relevance of lung overload to humans, and even to species other than laboratory rats and mice, is not clear. While it is likely to be of little relevance for most "real world" ambient exposures of humans, it is of concern in interpreting some long-term experimental exposure data and perhaps for humans' occupational exposure. In addition, relevance to humans is clouded by the suggestion that macrophage-mediated clearance is normally slower and perhaps less important in humans than in rats (Morrow, 1994), and that there can be significant differences in macrophage loading between species.

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 0.035, 0.220, and 1.890 mg/g of lung tissue, respectively. The alveolar clearance of those rats with the highest lung burden (1.890 mg/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 AMs. 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.

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). Application of the revised version 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 in the particle burden of the interstitial space, a compartment that is not available for macrophage-mediated clearance. The revised version of the POCK model is discussed in greater detail in the context of other dosimetry models below.

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 both sizes of particles were found to be phagocytized by AMs 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 poorly soluble particles of low toxicity (Lee et al., 1986, 1988; Ferin and 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. Particle overload only relates to poorly soluble articles of low toxicity. It must be noted, however, that some types of particles may be cytotoxic and impair clearance at lower lung burdens (e.g., silica may impair clearance at much lower lung burdens than DPM). 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.

3.4.3. 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 Type I cell population of the lung during lung overloading.

Another anatomical region in the lung that may be a slow clearing site is the interstitial compartment. Little is known about 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 could represent subcompartments of a more generalized slow clearing compartment.

Although these sites must be considered 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 aggregation 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.

3.5. MODELING THE DISPOSITION OF PARTICLES IN THE RESPIRATORY TRACT

3.5.1. Introduction

The biologic effects of inhaled particles are a function of their disposition. This, in turn, depends on their patterns of both deposition (i.e., the sites within which they initially come into contact with airway epithelial surfaces and the amount removed from the inhaled air at these sites) and clearance (i.e., the rates and routes by which deposited materials are removed from the respiratory tract). Removal of deposited materials involves the competing processes of macrophage-mediated clearance and dissolution-absorption. Over the years, mathematical models for predicting deposition, clearance and, ultimately, retention of particles in the respiratory tract have been developed. Such models help interpret experimental data and can be used to make predictions of deposition for cases where data are not available. A review of various mathematical deposition models was given by Morrow and Yu (1993) and in U.S. EPA (1996).

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.

3.5.2. Dosimetry Models for DPM

3.5.2.1. *Introduction*

Diesel particles are irregularly shaped aggregates with a mass median aerodynamic diameter (MMAD) of approximately 0.2 μm, formed from primary spheres 15-30 nm in

diameter. The primary sphere consists of a dense carbonaceous core (soot) on which various combustion-derived organic compounds, accounting for 10% to 30% of the particle mass, are adsorbed.

The extrapolation of toxicological results from laboratory animals to humans requires the use of dosimetry models for both species that include, first, the deposition of DPMs in various regions of the respiratory tract, and second, the transport and clearance of the particles from their deposited sites. Particles deposit by impaction, sedimentation, interception, and diffusion. The contribution from each mechanism is a function of particle size, lung structure, and size and breathing parameters. Because of the size of diesel particles, under normal breathing conditions most of this deposition takes place by diffusion, and the fraction of the inhaled mass that is deposited in the thoracic region is substantially similar for rats and humans. The clearance of particles takes place (1) by mechanical processes: mucociliary transport in the ciliated conducting airways and macrophage phagocytosis and migration in the nonciliated airways, and (2) by dissolution. The removal of the carbonaceous soot is largely by mechanical clearance, whereas the clearance of the adsorbed organics is principally by dissolution.

3.5.2.2. *Deposition Models*

Among deposition models that include aspects of lung structure and breathing dynamics, the most widely used have been typical-path or single-path models (Yu, 1978; Yu and Diu, 1983). The single-path models are based on an idealized symmetric geometry of the lung, assuming regular dichotomous branching of the airways and alveolar ducts (Weibel, 1963). They lead to modeling the deposition in an average regional sense for a given lung depth. Although the lower airways of the lung may be reasonably characterized by such a symmetric representation, there are major asymmetries in the upper airways of the tracheobronchial tree that in turn lead to different apportionment of airflow and particulate burden to the different lung lobes. The rat lung structure is highly asymmetric because of its monopodial nature, leading to significant errors in a single-path description. This is rectified in the multiple-path model of the lung that incorporates asymmetry and heterogeneity in lung branching structure, and calculates deposition at the individual airway level. This model has been developed for the rat lung by Anjilvel and Asgharian (1995) and, in a limited fashion because of insufficient morphometric data, for the human lung (Subramaniam et al., 1998; Yeh and Schum, 1980). Such models are particularly relevant for fine and ultrafine particles. However, models for clearance have not yet been implemented in conjunction with the use of the multiple-path model. Therefore, in this report we use only the single-path model in deposition calculations, specifically the works by Yu and Xu (1986) and Xu and Yu (1987).

3.5.2.3. *Physiologically Based Models for Clearance*

Several clearance models currently exist, and these differ significantly in the level of physiological detail that is captured in the model and in the uncertainties associated with the values of the parameters used. 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 the models. We compare four of the most widely discussed models below.

3.5.2.3.1. ***Two-compartment model.*** 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. 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.

3.5.2.3.2. ***Multicompartmental models.*** Strom et al. (1988) developed a multicompartmental model for particle retention that partitioned the alveolar region into two compartments on the basis of the physiology of clearance. The alveolar region has a separate compartment for sequestered macrophages, which corresponds to phagocytic macrophages that are heavily laden with particles and clustered, and therefore have significantly lowered mobility. The model has 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 dictates sequestration of particles and their eventual transfer to the lymph nodes. The transport rates between various compartments were obtained by fitting the calculated results to lung and lymph node burden experimental data for both exposure and postexposure periods. Since the number of fitted parameters was large, the model is not likely to provide unique solutions that would simulate experimental data from various sources and for different exposure scenarios. For the same reason, it is not readily possible to use this model for extrapolating to humans.

3.5.2.3.3. ***POCK model.*** Stöber and co-workers have worked extensively in developing models for estimating retention and clearance of biologically insoluble, respirable particles in the lung.

Their most recent work (1994), a revised version of the POCK (physiologically oriented multicompartmental kinetic) model, is a rigorous attempt to incorporate most of the physiologically known aspects of alveolar clearance and retention of inhaled insoluble particles. Their multicompartmental kinetics model has five subcompartments. The transfer of particles between any of the compartments within the alveolar region is macrophage-mediated. There are two compartments that receive particles cleared from the alveolar regions: the tracheobronchial tract and the lymphatic system.

The macrophage pool includes both mobile and particle-laden, immobilized macrophages. 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. Sequestration of those macrophages heavily loaded with a particle burden close to a volume load capacity is treated in a sophisticated manner by approximating the particle load distribution in the macrophages. The macrophage pool is compartmentalized in terms of numbers of macrophages that are subject to discrete particle load intervals. Upon macrophage death, the phagocytized particle is released back to the alveolar surface; thus phagocytic particle collection competes to some extent with this release back to the alveolar surface. This recycled particle load is also divided into particle clusters of size intervals defining a cluster size distribution on the alveolar surface. The model yields a time-dependent frequency distribution of loaded macrophages that is sensitive to both exposure and recovery periods in inhalation studies.

The POCK 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.

While the POCK model is the most sophisticated in the physiological complexity it introduces, it suffers from a major disadvantage. Experimental retention studies provide data on total alveolar and lymph node mass burdens of the particles as a function of time. The relative fraction of the deposit between the alveolar subcompartments in the Stöber model therefore cannot be obtained experimentally; the model thus uses a large number of parameters that are simultaneously fit to experimental data. 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. Thus the over-parameterization in the model leads to the problem that the model may not provide a unique solution that may be used for a variety of exposure scenarios, and for the same reason, cannot be used for extrapolation to humans. Stöber et al. have not developed an equivalent model for humans; therefore the use of their model in our risk assessment for diesel is not attempted.

3.5.2.3.4. ***Yu-Yoon model.*** Yu and Yoon (1990), on the other hand, have developed a three-compartment lung model that consists of tracheobronchial (T), alveolar (A), and lymph node (L) compartments (Appendix B, Figure B-1) and, in addition, considered filtration by a nasopharyngeal or head (H) compartment. Absorption by the blood (B) and gastrointestinal (G) compartments was also considered. While the treatment of alveolar clearance is physiologically less sophisticated than that of the Stöber et al. model, the Yu-Yoon model provides a more comprehensive treatment of clearance by including systemic compartments and the head, and including the clearance of the organic components of DPM in addition to the insoluble carbon core.

The tracheobronchial compartment is important for short-term considerations, while long-term clearance takes place via the alveolar compartment. In contrast to the Stöber and Strom approaches, the macrophage compartment in the Yu-Yoon model contains all of the phagocytized particles; that is, there is no separate (and hypothetical) sequestered macrophage subcompartment. Instead, in order to progress beyond the classical retention model (International Commission on Radiological Protection, 1979), Yu and Yoon have addressed the impairment of long-term clearance (the overload effect) by using a set of variable transport rates for clearance from the alveolar region as a function of the mass of DPM in the alveolar compartment. A functional relationship for this was derived mathematically (Yu et al., 1989) based upon Morrow's hypothesis for the overload effect that we discussed earlier in the section on pulmonary overload. The extent of the impairment depends on the initial particle burden, with greater particulate concentration leading to slower clearance.

DPM are treated as composed of three material components: an insoluble carbonaceous core, slowly cleared organics (10% particle mass), and fast-cleared organics (10% particle mass). Such a partitioning of organics was based on observations that the retention of particle-associated organics in lungs shows a biphasic decay curve (Sun et al., 1984; Bond et al., 1986). For any compartment, each of these components has a different transport rate. The total alveolar clearance rate of each material component is the sum of clearance rates of that material from the alveolar to the tracheobronchial, lymph, and blood compartments. In the Strom and Stöber models discussed above, the clearance kinetics of DPM were assumed to be entirely dictated by that of the insoluble carbon core. For those organic compounds that get dissociated from the carbon core, clearance rates are likely to be very different, and some of these compounds may be metabolized in the pulmonary tissue or be absorbed by blood.

The transport rates were derived from experimental data for rats using several approximations. The transport rates for the carbonaceous core and the organic components were derived by fitting to data from separate experiments. Lung and lymph node burdens from the experiment of Strom et al. (1988) were used to determine the transport rate of the carbon core. The Yu-Yoon model incorporates the impairment of clearance by including a mass dependency

in the transport rate. This mass dependency is easily extracted because the animals in the experiment were killed over varying periods following the end of exposure.

It was assumed that the transport rates from the alveolar and lymph compartments to the blood were equal and independent of the particulate mass in the alveolar region. The clearance rates of particle-associated organics for rats were derived from the retention data of Sun et al. (1984) for benzo[a]pyrene and the data of Bond et al. (1986) for nitropyrene adsorbed on diesel particles.

3.5.2.4. *Model Assumptions and Extrapolation to Humans*

The Yu-Yoon approach takes the perspective that parsimonious models are to be preferred in order to enable experimental validation and extrapolation from rats to humans.

Yu and Yoon make two important assumptions to carry out the extrapolation in the light of inadequate human data. First, the transport rates of organics in the DPM do not change across species. This is based upon lung clearance data of inhaled lipophilic compounds (Schanker et al., 1986), where the clearance was seen to be dependent on the lipid/water partition coefficient. In contrast, the transport rate of the carbon core is considered to be significantly species-dependent (Bailey et al., 1982). DPM clearance rate is determined by two terms in the model (see equation C-82). The first, corresponding to macrophage-mediated clearance, is a function of the lung burden, and is assumed to vary significantly across species. The second term, a constant, corresponding to clearance by dissolution, is assumed to be species-independent. The mass-dependent term for humans is assumed to vary in the same proportion as in rats under the same unit surface particulate dose. The extrapolation is then achieved by using the data of Bailey et al. (1982) for the low lung burden limit of the clearance rate. This value of 0.0017/day was lower than the rat value by a factor of 7.6. This is elaborated further in Appendix C. Other transport rates that have lung burden dependence are extrapolated in the same manner.

The Bailey et al. experiment, however, used fused monodisperse aluminosilicate particles of 1.9 and 6.1 μm aerodynamic diameters. Yu and Yoon have used the longer of the half-times obtained in this experiment; in using such data for diesel soot particles 0.2 μm in diameter, they have assumed the clearance of insoluble particles to be independent of size over this range. This appears to be a reasonable assumption since the linear dimensions of an alveolar macrophage is significantly larger, roughly 10 μm (Yu et al., 1996). However, Snipes (1979) has reported a clearance rate (we convert here from their half-life values) of 0.0022/day for 1 and 2 μm particles but a higher value of 0.0039/day for 0.4 μm particles. In the absence of reliable data for 0.2 μm particles, clearance rate pertaining to a much larger particle size is being used. Although such a choice may underestimate the correct clearance rate for DPM, the resulting error in the human equivalent concentration is likely to be only more protective of human health. Long-term clearance rates for particle sizes more comparable to DPM are available, e.g., iron oxide and

polystyrene spheres (Waite and Ramsden, 1971; Jammet et al., 1978), but these data show a large range in the values obtained for half-lives or are based upon a very small number of trials, and therefore compare unfavorably with the quality of data from the Bailey experiment.

The deposition fractions of particulate matter in the pulmonary and tracheobronchial regions of the human lung remain relatively unchanged over the particle size range between 0.2 and 1.0 μm. Since the clearance of insoluble particles is also likely to remain the same over this range, the dosimetry results in this report for the carbon core component of DPM could also be extended to other particles in this size range within the PM2.5. For particle diameters between 1.0 and 3.5 μm, the deposition fraction in the pulmonary region increases significantly (Yu and Diu, 1983), so the diesel model will not be applicable for particles in this range without changing the value for the deposition fractions.

Although there was good agreement between experiment and calculated results, this agreement follows a circular logic (as adequately pointed out by Yu and Yoon [1990]) because the same experimental data figured into the derivation of transport rates used in the model. Nevertheless, while this agreement is not a validation, it provides an important consistency check on the model. Thus, the model awaits further experimental data for a reasonable validation.

The model showed that at low lung burdens, alveolar clearance is dominated by mucociliary transport to the tracheobronchial region, and at high lung burdens, clearance is dominated by transport to the lymphatic system. The head and tracheobronchial compartments showed quick clearance of DPM by mucociliary transport and dissolution. Lung burdens of both the carbon core and organics were found to be greater in humans than in rats for similar periods of exposure.

The Yu-Yoon publication provides a parametric study of the dosimetry model, examining variation over a range of exposure concentrations, breathing scenarios and ventilation parameters, particle mass median aerodynamic diameters, and geometric standard deviations of the aerosol distribution. It examines how lung burden varies with age for exposure over a life span, provides dosimetry extrapolations to children, and examines changes in lung burden with lung volume. The results showed that children would exhibit more diminished alveolar clearance of DPM at high lung burden than adults when exposed to the equal concentrations of DPM. These features make the model easy to use in risk assessment studies. We refer the reader to Appendix C for further details on the model and for analyses of the sensitivity of the model to change in parameter values.

The Yu-Yoon model presents some uncertainties in addition to those discussed earlier in the context of particle size dependence of clearance rate. The Yu and Yoon report underwent extensive peer review; we list below the most important among the model uncertainties discussed by the review panel. The experimental data used by the Yu-Yoon model for adsorbed organics used passively adsorbed radiolabeled compounds as surrogates for combustion-derived organics.

These compounds may adhere differently to the carbon core than those formed during combustion. Yu and Yoon have estimated that slowly cleared organics represent 10% of the total particle mass; the actual figure could be substantially less; the reviewers estimate that the amount of tightly bound organics is probably only 0.1% to 0.25% of the particle mass.

The model was based upon the experimental data of Strom et al. (1988) where Fischer-344 rats were exposed to DPM at a concentration of 6.0 mg/m^3 for 20 hours/day and 7 days/week for periods ranging from 3 to 84 days. Such exposures lead to particle overload effects in rats, whereas human exposure patterns are usually of much lower levels at which overload will not occur. Secondly, human exposures are not likely to be continuous, but most likely over brief periods of time. Parameters obtained by fitting to data under the conditions of the experimental scenario for rats may not be optimal for the human exposure and concentration of interest.

The extrapolation of retained dose from rats to humans assumed that the macrophage-mediated mechanical clearance of the DPM varies with the specific particulate dose to the alveolar surface in the same proportion in humans and in rats, whereas clearance rates by dissolution were assumed to be invariant across species. This assumption has not been validated.

3.5.3. Deposition of Organics

Using the data presented by Xu and Yu (1987), it is possible to calculate the total mass of DPM, as well as the total organic mass and specific carcinogenic PAHs deposited in the lungs of an individual exposed to DPM. For example, the annual deposition of DPM in the lungs of an individual exposed continuously to 1 μg/m^3 DPM can be estimated to be about 420 μg. About 0.7% of particle mass consists of PAHs (see Section 2.2.6.2, Chapter 2) for a total of 2.94 μg. Of this amount, the deposited mass of nitro-polycyclic aromatic compounds based on data by Campbell and Lee (1984) would equal 37 ng, while the deposited mass of 7 PAHs that tested positive in cancer bioassays (U.S. EPA, 1993), and measured by Tong and Karasek (1984), would range from 0.16 to 0.35 μg. While these amounts are very small, exposure concentrations are often greater than 1 μg/m^3, and deposition in humans can be expected to be concentrated at limited sites, especially at the bifurcations of the small bronchi.

3.6. 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. Since then evidence has been presented that carbon black, lacking an organic component, is capable of inducing lung cancer at exposure concentrations sufficient to induce lung particle overload. This suggested that the insoluble carbon core of the particle may be of greater importance for the pathogenic and carcinogenic processes observed in

the rat inhalation studies conducted at high exposure concentrations. (See Chapter 7 for a discussion of this issue.) Nevertheless, because lung tumor induction was reported in epidemiology studies at exposure levels unlikely to induce lung particle overload, it is reasonable to assume that organic compounds play a role.

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. In order to reach the target site, elution from the particle surface is necessary followed by diffusion and uptake by the target cell. Metabolism to an active form by either the phagocytes or the target cells is also required for activity of many of the compounds present.

3.6.1. In Vivo Studies

3.6.1.1. *Laboratory Investigations*

Several studies reported on the retention of particle-adsorbed organics following administration to various rodent species. In studies reported by Sun et al. (1982, 1984) and Bond et al. (1986), labeled organics were deposited on diesel particles following heating to vaporize the organics originally present. Sun et al. (1982) compared the disposition of either pure or diesel particle-adsorbed benzo[a]pyrene B[a]P following nose-only inhalation by F344 rats. About 50% of particle-adsorbed B[a]P was cleared with a half-time of 1h predominantly by mucociliary clearance. The long-term retention of particle-adsorbed ^{3}H-B[a]P (18 days) was approximately 230-fold greater than that for pure ^{3}H-B[a]P (Sun et al., 1982). At the end of exposure, about 15% of the ^{3}H label was found in blood, liver, and kidney. Similar results were reported in a companion study by Bond et al. (1986), and by Sun et al. (1984) with another PAH, 1-nitropyrene, except retention half-time was 36 days.

Ball and King (1985) studied the disposition and metabolism of intratracheally instilled ^{14}C-labeled 1-NP (>99.9% purity) coated onto DPM. About 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. 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.

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 containing 1.03 μg B[a]P/g particles were supplemented with exogenous ^{3}H-B[a]P to provide 2.62 μg B[a]P/g of exhaust particles. In vitro analysis indicated that the supplemented B[a]P eluted from the particles at the same rate as the original B[a]P. Twenty-four hours after intratracheal instillation in Sprague-Dawley rats, 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), possibly because smaller amounts of B[*a*]P adsorbed on the particles, resulting in stronger binding. 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. Results of these studies showed that the retention of organics in the lungs is increased considerably when organics are adsorbed to diesel particles. Because retention time is very short following exposure to the pure compounds, it can be concluded that the increased retention time is primarily the result of continued binding to the particles. The detection of labeled compounds in blood, distant organs, urine, and bile as well as the trachea, however, provides evidence that at least some of the organics are eluted from the particles following deposition in the lungs.

3.6.1.2. *Studies in Occupationally Exposed Humans*

DNA adduct induction in the lungs of experimental animals exposed to diesel exhaust have been measured in a number of animal experiments (see World Health Organization [1996] for a review). Such studies, however, provide limited information regarding bioavailability of organics, since positive results may well have been related to factors associated with lung particle overload. In fact, Bond et al. (1990) reported that carbon black, which is virtually devoid of organics, is capable of inducing DNA adducts in rats at lung overload doses.

On the other hand, DNA adduct formation and/or mutations in blood cells following exposure to DPM, especially at levels insufficient to induce lung overload, can be presumed to be the result of organics diffusing into the blood. Hemminki et al. (1994) reported increased levels of DNA adducts in lymphocytes of bus maintenance and truck terminal workers. Österholm et al. (1995) studied mutations at the hprt-locus of T-lymphocytes in bus maintenance workers. Although they were unable to identify clearcut exposure-related differences in types of mutations, adduct formation was significantly increased in the exposed workers. Nielsen et al. (1996) reported significantly increased levels of lymphocyte DNA adducts, hydroxyvaline adducts in hemoglobin, and 1-hydroxypyrene in urine of garage workers exposed to diesel exhaust.

3.6.2. In Vitro Studies

3.6.2.1. *Extraction of Diesel Particle-Associated Organics by Biological Fluids*

In vitro extraction of organics in biological fluids can be estimated by measurement of mutagenic activity. Using this approach, Brooks et al. (1981) reported extraction efficiencies of only 3% to 10 % that of dichloromethane following DPM incubation in lavage fluid, serum, saline, albumin, dipalmitoyl lecithin, or dichloromethane. Moreover, extraction efficiency did

not increase with incubation time up to 120 h. Similar findings were reported by King et al. (1981). In the latter study, lung lavage fluid and lung cytosol fluid extracts of DPM were not mutagenic. Serum extracts of DPM did exhibit some mutagenic activity, but 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.

Despite the apparent inability of biological fluids to extract organics in vitro, their effectiveness in vivo remains equivocal because of differing conditions. 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. Finally, except under very high exposure concentrations, few particles escape phagocytosis and possible intracellular extraction.

3.6.2.2. *Extraction of Diesel Particle-Associated Organics by Lung Cells and Cellular Components*

A more likely means by which organic carcinogens (e.g., PAHs) may be extracted from DPM and metabolized in the lung is either particle dissolution or extraction of organics from the particle surface within the phagolysosomes of AMs and other lung cells. This mechanism presupposes that the particles are internalized. 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 DPM (e.g., B[a]P) may be extracted in the phagolysosomes (Bond et al., 1983). Whatever the mechanism, assuming elution occurs, the end result is a prolonged exposure of the respiratory epithelium to low concentrations of carcinogenic agents.

Early studies by King et al. (1981) found that when pulmonary alveolar macrophages were incubated with DPM, amounts of organic compounds and mutagenic activity decreased

measurably from the amount originally associated with the particles, suggesting that organics were removed from the phagocytized particles. Leung et al. (1988) studied the ability of rat lung and liver microsomes to facilitate transfer and metabolism of B[*a*]P from diesel particles. ^{14}C-B[*a*]P coated diesel particles, previously extracted to remove the original organics present, were incubated with liver or lung microsomes. About 3% of the particle adsorbed B[*a*]P was transferred to the lung microsomes within 2 h. Of this amount about 1.5% was metabolized, for a total of about 0.05% of the B[*a*]P adsorbed to the DPM. While transformation is slow, because of long retention half-lives of particles in humans the fraction eluted and metabolized may well be significant.

In analyzing phagolysosomal dissolution of various ions from particles in the lungs of Syrian golden hamsters, however, 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.

3.6.3. Modeling Studies

Gerde et al. (1991a,b) described a model simulating the effect of particle aggregation and PAH content on the rate of PAH release in the lung. According to this model, particle aggregation will occur with high exposure concentrations, resulting in a slow release of PAHs with prolonged exposure to surrounding tissues. However, large aggregates of inert dust are unlikely to form at doses typical of human exposures. Inhaled particles, at low concentrations, are more likely to deposit and react with surrounding lung medium without interference from other particles. The model predicts that, under low-dose exposure conditions more typical in humans, particle-associated organics will be released more rapidly from the particles because they are not aggregated. Sustained exposure of target tissues to PAHs will result from repeated exposures, not from increased retention due to association of PAHs with carrier particles. This distinction is important because at low doses PAH exposure and lung tumor formation should occur at sites of deposition rather than retention as occurs with high doses.

The site of release of PAHs influences effective dose to the lungs because, as noted previously, at least some free organic compounds such as B[*a*]P deposited in the lungs are rapidly absorbed into the bloodstream. Gerde et al. (1991b) predicted lipophilic PAHs would be retained in the alveoli less than 1 min, whereas they may be retained for hours in the bronchi. These predictions were based on an average diffusion distance to capillaries of only about 0.5 μm in the alveoli, whereas in the bronchi it probably exceeds 50 μm. An experimental study by Gerde et al. (1999) provided support for this prediction. Beagle dogs were exposed to ^{3}H-B[*a*]P adsorbed on the carbonaceous core of diesel particles at a concentration of 15 μg B[*a*]P/gm

particles. A rapidly eluting fraction from particles deposited in the alveoli was adsorbed into the bloodstream and metabolized in the liver. The rapidly eluting fraction from particles deposited in the conducting airways, however, was to a large extent retained and metabolized in airway epithelium.

Nikula et al. (1997) reported that 52% of DPM deposited in the lungs of Cynomolgus monkeys chronically inhaling diesel exhaust were found in the interstitium of small airways, compared with 27% in rats (Nikula et al., 1997). This is primarily due to a lack of respiratory bronchioles in the rat. Because lung structure is similar in monkeys and humans, a significantly greater percentage of DPM matter can be predicted to deposit in small airway branches of humans. Overall, bioavailability of organics in humans is expected to be greatest at bifurcations of small airways because they are a major site of particle deposition, have a longer residence time for eluted organics in airways than alveoli, allow time for uptake and metabolism by airway epithelium, and predict more rapid elution from particles at ambient exposure concentrations.

Overall, the results of studies presented in Section 3.6 provide evidence that at least some of the organic matter adsorbed to DPM deposited in the lungs is eluted. However, the percentage taken up and metabolized to an active form by target cells is uncertain. Organics eluted from particles deposited in alveoli are likely to rapidly enter the bloodstream and pose little risk for induction of lung pathology and/or cancer. Risk of harmful effects for particles deposited in the small airways is predicted to be greater because solubilized organic compounds will be retained longer, allowing for metabolism by epithelial cells lining the airways. Since the deposition in small airways occurs primarily at bifurcations, localized higher concentrations will occur. At present, unfortunately, the available data are insufficient to accurately model the effective dose of organics in the respiratory tract of humans or animals exposed to diesel exhaust, especially at specific target sites such as small airway branchings.

3.7. SUMMARY

The most consistent historical measure of dose for diesel emissions is DPM in units of μg particles/m^3. With the assumption that all components of diesel emissions (e.g., organics in the form of volatilized liquids or gases) are present in proportion to the DPM mass, DPM can be used as the basic dosimeter for effects from various scenarios including acute and chronic exposures, as well as for different endpoints such as irritation, fibrosis, or even cancer. There is, however, little evidence currently available to prove or refute DPM as being the most appropriate dosimeter.

The DPM dose to the tissue is related to the extent of the deposition and clearance of DPM. Diesel exhaust particles may deposit throughout the respiratory tract via sedimentation or diffusion, with the latter being prevalent in the alveolar region. Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely or may be translocated to

other sites by regionally distinct processes that can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles via mucociliary transport). With insoluble or poorly soluble particles such as DPM, clearance by dissolution is insignificant compared to the rate of clearance as an intact particle. Another mechanism that can affect retention of DPM is endocytosis by alveolar lining cells that, in turn, can lead to the accumulation of DPM in the interstitial compartment of the lung and subsequent translocation of DPM to lymph nodes. For poorly soluble particles such as DPM, species-dependent rate constants exist for the various clearance pathways that can be modified by factors such as respiratory tract disease.

In rats, prolonged exposure to high particle concentrations may be associated with what is termed particle overload. This condition is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway, occurring in rats when deposition approaches 1 mg particles/g lung tissue. The relevance of lung overload to humans, and even to species other than laboratory rats and mice, is problematic. Relevance to humans is further clouded by the suggestion that macrophage-mediated clearance is normally slower and perhaps less important in humans than in rats. Whereas such clearance is likely to be of little relevance for most "real-world" ambient exposures of humans, it is of concern in interpreting some long-term experimental exposure data and perhaps for some human occupational exposures.

Extrapolation of toxicological results from laboratory animals to humans to obtain an HEC requires the use of a dosimetry model incorporating critical aspects of both species that include (1) the deposition of DPM in various regions of the respiratory tract, and (2) the transport and clearance of the particles from their deposited sites. Review and evaluation of the models available led to the choice of the Yu and Yoon (1990) model. This model has a three-compartment lung consisting of tracheobronchial, alveolar, and lymph node compartments and, in addition, considers filtration by a nasopharyngeal or head compartment. Absorption by the blood and gastrointestinal compartments was also considered. In addition, the model treats DPM as being composed of the insoluble carbonaceous core, slowly cleared organics, and fast-cleared organics. Major assumptions made in this model include that transport rates of organics in DPM do not change across species and that the transport rate of the carbonaceous core is species dependent such that the clearance varies with specific dose to the alveolar surface in the same proportion in humans and in rats. This model was used to project HECs from concentrations used in experimental animal exposures. Use of HECs partially obviates the need for an animal-to-human uncertainty factor, as explained in Chapter 9.

The degree of bioavailability of the organic fraction of DPM is still somewhat uncertain. However, reports of DNA induction in occupationally exposed workers as well as results of animal studies using radiolabeled organics deposited on diesel particles indicate that at least a

fraction of the organics present are eluted prior to particle clearance. In addition, data have been presented indicating that a greater percentage of diesel particles are deposited in the branching of small airways of laboratory primates, and presumably of humans, than in those of rats. Carcinogenic organics eluted in this region remain in the lung long enough to be metabolized to an active form. Some of the toxicologically significant compounds, however, do not require metabolic activation. While adequate quantitative data are lacking, they do suggest the likely involvement of particle-associated organics in the carcinogenic process.

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4. MUTAGENICITY OF DIESEL EXHAUST

Since 1978, more than 100 publications have appeared in which genotoxicity assays were used with diesel emissions, volatile and particulate fractions (including extracts), or individual chemicals found in diesel emissions. Although most of the studies deal with 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. As indicated in Chapter 2, the number of chemicals in diesel emissions is very large. Many of these (e.g. PAHs and nitro-PAHs) have been determined to exhibit mutagenic activity in a variety of assay systems. Because of the limited and uncertain role of the individual chemicals in either the cancer or noncancer effects of diesel emissions, discussion of those data are not included. Also, several review articles, some containing more detailed descriptions of the available studies, are available (International Agency for Research on Cancer, 1989) (Claxton, 1983; Pepelko and Peirano, 1983; Shirnamé-Moré, 1995). The proceedings of several symposia on the health effects of diesel emissions (U.S. EPA, 1980; Lewtas, 1982; Ishinishi et al., 1986) are also available. An understanding of diesel exhaust mutagenicity is important to the cancer health effects and dose-response chapters, Chapters 7 and 8, respectively.

4.1. 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). In a 1983 review, Claxton discussed a number of factors that affected the mutagenic response in Salmonella assays. Citing the data from the Huisingh et al. (1978) study, the author noted that the mutagenic response could vary by a factor of 100 using different fuels in a single diesel engine. More recently, Crebelli et al. (1995) used Salmonella to examine the effects of different fuel components. They reported that while mutagenicity was highly dependent on aromatic content, especially di- or triaromatics, there was no clear effect of sulfur content. Later, Sjögren et al. (1996), using multivariate statistical methods with 10 diesel fuels, concluded that the most influential chemical factors in Salmonella mutagenicity were sulfur contents, certain polycyclic aromatic hydrocarbons (PAHs) (1-nitropyrene), and naphthenes.

Matsushita et al. (1986) tested particle-free diesel exhaust gas and a number of benzene nitro-derivatives and 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. Barfknecht 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 more than 40% of the observed activity.

Morimoto et al. (1986) injected DPM extracts (250 to 4000 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 2000 and 4000 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.

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.

Driscoll et al. (1996) exposed Fischer 344 male rats to aerosols of carbon black (1.1, 7.1, and 52.8 mg/m^3) or air for 13 weeks (6 h/day, 5 days/week) and measured *hprt* mutations in alveolar type II cells in animals immediately after exposure and at 12 and 32 weeks after the end of exposure. Both the two higher concentrations resulted in significant increases in mutant frequency. While the mutant frequency from the 7.1 mg/m^3 group returned to control levels by 12 weeks, the mutant frequency of the high exposure group was still higher than controls even after 32 weeks. Carbon black particles have very little adsorbed PAHs, hence a direct chemically-induced mechanism is highly unlikely. The authors suggested that the likely explanation for the observed increases was persistent pulmonary inflamation and hyperplasia.

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.

Hou et al. (1995) measured DNA adducts and *hprt* mutations in 47 bus maintenance workers and 22 control individuals. All were nonsmoking men from garages in the Stockholm area and the exposed group consisted of 16 garage workers, 25 mechanics, and 6 other garage workers. There were no exposure data, but the three groups were considered to be of higher to lower exposure to diesel engine exhaust. Levels of DNA adducts determined by ^{32}P-postlabeling were significantly higher in workers than in controls (3.2 versus 2.3 × 10^{-8}), but *hprt* mutant frequencies were not different (8.6 versus 8.4 × 10^{-6}). Both adduct level and mutagenicity were highest among the 16 most exposed workers and mutant frequency was significantly correlated with adduct level. All individuals were genotyped for glutathione transferase GSTM1 and aromatic amino transferase NAT2 polymorphism. Neither GSTM1 nulls nor NAT2 slow acetylators exhibited effects on either DNA adducts or *hprt* mutant frequencies.

4.2. 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).

Gu et al. (1992) reported that DEP dispersed in an aqueous mixture containing dipalmitoyl lecithin (DPL), a component of pulmonary surfactant or extracted with dichloromethane (DCM) induced similar responses in micronucleus tests in Chinese hamster V79 and CHO cell cultures. After the samples were separated into supernatant and sediment fractions, mutagenic activity was confined to the sediment fraction of the DPL sample and the supernatant of the DCM sample. These findings suggest that the mutagenic activity of DEP inhaled into the lungs could be made bioavailable through solubilization and dispersion nature of pulmonary surfactants, but the application of these in vitro findings to conditions in the human lung remains to be studied.

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.

4.3. 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 (control values were 9.2%, 14.9%, and 27.8% in the three laboratories). Conversely, male Chinese hamsters exposed for 6 months (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.

4.4. 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 months. 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.

Application of mutagenicity data to the question of the potential carcinogenicity of diesel emissions is based on the premise that genetic alterations are found in all cancers and that several of the chemicals found in diesel emissions possess mutagenic activity in a variety of genetic assays. These genetic alterations can be produce by gene mutations, deletions, translocations, aneuploidy, or amplification of genes, hence no single genotoxicity assay should be expected to either qualitatively or quantitatively predict rodent carcinogenicity. With diesel emissions or other mixtures, additional complications arise because of the complexity of the material being tested. Exercises that combined the Salmonella mutagenic potency with the total concentration of mutagenic chemicals deposited in the lungs could not account for the observed tumor incidence in exposed rats (Rosenkranz, 1993,Goldstein, et al. 1998). Additionally, it appears that the some of 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).

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5. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

The objective of this chapter is to report, evaluate, and interpret health effects other than cancer that have been associated with inhalation exposure to diesel exhaust. Data on this class of health effects of diesel exhaust have been obtained from diverse human, laboratory animal, and in vitro test systems. The human studies comprise both occupational and human experimental exposures, the former consisting of exposure to diesel exhaust in the occupational environment and the latter consisting of exposure to diluted diesel exhaust or diesel particulate matter (DPM) under controlled conditions. The laboratory animal studies consist of both acute and chronic exposures of laboratory animals to diesel exhaust or DPM. Diverse in vitro test systems composed of human and laboratory animal cells treated with DPM or components of DPM have also been used to investigate the effects of DPM at the cellular and molecular levels. The noncancer health effects of ambient particulate matter, which is composed in part of DPM, as well as the potential mechanisms underlying these effects, have been reviewed previously (Health Effects Institute, 1995; U.S. EPA, 1996).

5.1. HEALTH EFFECTS OF WHOLE DIESEL EXHAUST

5.1.1. Human Studies

5.1.1.1. *Short-Term Exposures*

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

inadequate to generalize about the human response. To date, no well-controlled chamber study has been conducted using methodologies for assessing subtle lung inflammatory reactions.

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

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

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

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

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

The role of antioxidant defenses in protecting against acute diesel exhaust exposure has been studied. Blomberg et al. (1998) investigated changes in the antioxidant defense network within the respiratory tract lining fluids of human subjects following diesel exhaust exposure. Fifteen healthy, nonsmoking, asymptomatic subjects were exposed to filtered air or diesel exhaust (DPM 300 mg/m^3) for 1 hr on two separate occasions at least three weeks apart. Nasal lavage fluid and blood samples were collected prior to, immediately after, and 5 ½ hr post exposure. Bronchoscopy was performed 6 hr after the end of diesel exhaust exposure. Nasal lavage ascorbic acid concentration increased 10-fold during diesel exhaust exposure, but returned to basal levels 5.5 hr post-exposure. Diesel exhaust had no significant effects on nasal lavage uric acid or GSH concentrations, and did not affect plasma, bronchial wash, or bronchoalveolar lavage antioxidant concentrations, nor malondialdehyde or protein carbonyl concentrations. The authors concluded that the physiological response to acute diesel exhaust exposure is an acute increase in the level of ascorbic acid in the nasal cavity, which appears to be sufficient to prevent further oxidant stress in the respiratory tract of healthy individuals.

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

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

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

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

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

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

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

formaldehyde. The investigators reported a statistically significant decline in FVC and FEV_1 over the workshift in both the diesel-exposed and comparison groups. Current smokers had greater decrements in FVC, FEV_1, and FEF_{50} than ex-smokers and nonsmokers. There was a marked disparity between the ages and the time spent underground for the two study groups. Diesel-exposed miners were about 15 years younger and had worked underground for 15 fewer years (4.8 versus 20.7 years) than miners not exposed to diesel exhaust. The significance 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. (1979) measured preshift FEV_1 and FVC in 187 salt miners and obtained peak flow forced expiratory flow rates at 25, 50, and 75% of FVC (FEF_{25}, FEF_{50}, or FEF_{75}). Postshift pulmonary function values were determined from total lung capacity and flows at preshift percentages of FVC. The miners were exposed to mean NO_2 levels of 1.5 ppm and mean respirable particulate levels of 0.7 mg/m^3. No statistically significant changes were found between changes in pulmonary function and in NO_2 and respirable particles combined. Slopes of the regression of NO_2 and changes in FEV_1, FEF_{25}, FEF_{50}, and FEF_{75} were significantly different from zero. The authors concluded that these small reductions in pulmonary function were attributable to variations in NO_2 within each of the five salt mines that contributed to the cohort.

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

Workers who had respiratory symptoms had slightly greater but statistically insignificant reductions in FEV_1 and FEF_{50}.

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

5.1.1.1.3. ***Immunological effects***. The potential for DPM to cause immunologic changes has been investigated in several studies. Wade and Newman (1993) reported that diesel exhaust can induce reactive airway disease in railroad workers. In that study, three workers were identified who developed asthma following either a single exposure or a series of short-term exposures to high concentrations of diesel exhaust. 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.

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

In an attempt to evaluate the potential allergenic effects of DPM in humans Diaz-Sanchez and associates carried out a series of clinical investigations. In the first of these (Diaz-Sanchez et al., 1994), healthy human volunteers were challenged by spraying either saline or 0.30 mg DPM into their nostrils. This dose was considered equivalent to total exposure on 1-3 average days in Los Angeles, but could occur acutely in certain nonoccupational settings such as sitting at a busy bus stop or in an express tunnel. Enhanced IgE levels were noted in nasal lung lavage cells in as little as 24 h, with peak production observed 4 days after DPM challenge. The effects seemed to be somewhat isotype-specific, because in contrast to IgE results, DPM challenge had no effect on the levels of IgG, IgA, IgM, or albumin. The selective enhancement of local IgE production was demonstrated by a dramatic increase in IgE-secreting cells.

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

The ability of DPM to act as an adjuvant to the ragweed allergen Amb a I was also examined by nasal provocation in ragweed-allergic subjects using 0.3 mg DPM, Amb a I, or both (Diaz-Sanchez et al., 1997). Although allergen and DPM each enhanced ragweed-specific IgE,

DPM plus allergen promoted a 16-times greater antigen-specific IgE production. Nasal challenge with DPM also influenced cytokine production. Ragweed challenge resulted in a weak response, DPM challenge caused a strong but nonspecific response, and allergen plus DPM caused a significant increase in the expression of mRNA for TH0 and TH2-type cytokines (IL-4, IL-5, IL-6, IL-10, IL-13), with a pronounced inhibitory effect on IFN-γ gene expression. The author concluded that DPM can enhance B-cell differentiation and, by initiating and elevating IgE production, may be a factor in the increased incidence of allergic airway disease.

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

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

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

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

The mechanisms underlying DPM-induced injury to airway cells were investigated in human bronchial epithelial cells (HBEC) in culture (Bayram et al., 1998). HBEC from bronchial explants obtained at surgery were cultured and exposed to DPM (10-100 μg/mL) or to a filtered solution of DPM (50 μg/mL), and the effects on permeability, ciliary beat frequency (CBF), and release of inflammatory mediators were observed. DPM and filtered solution of DPM significantly increased the electrical resistance of the cultures but did not affect movement of bovine serum albumin across cell cultures. DPM and filtered DPM solution significantly attenuated the CBF of these cultures and significantly increased the release of IL-8. DPM also increased the release by these cultures of GM-CSF and soluble intercellular adhesion molecule-1 (sICAM-1). The authors concluded that exposure of airway cells to DPM may lead to functional changes and release of proinflammatory mediators and that these effects may influence the development of airway disease.

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

Devalia et al. (1999) investigated the potential sensitivity of bronchial epithelial cells (HBEC) biopsied from atopic mild asthmatic patients and non-atopic nonasthmatic subjects to DPM. HBEC from asthmatic patients constitutively released significantly greater amounts of IL-8, GM-CSF, and sICAM-1 than HBEC from nonasthmatic subjects. RANTES was only released by HBEC of asthmatic patients. Incubation of the asthmatic cultures with 10 µg/mL DPM significantly increased the release of IL-8, GM-CSF, and sICAM-1 after 24 h. In contrast, only higher concentrations (50-100 µg/mL DPM) significantly increased the release of IL-8 and

GM-CSF from HBEC of nonasthmatics. The authors conclude that the increased sensitivity of the airways of asthmatics to DPM may be, at least in part, a consequence of greater constitutive and DPM-induced release of specific pro-inflammatory mediators from bronchial epithelial cells.

Boland et al. (1999) compared the biological effects of carbon black and DPM collected from catalyst- and noncatalyst-equipped diesel vehicles in cultures of both human bronchial epithelial cells (16HBE14o-) and human nasal epithelial cells. Transmission electron microscopy indicated that DPM was phagocytosed by epithelial cells and translocated through the epithelial cell sheet. The time and dose dependency of phagocytosis and its nonspecificity for different particles (DPM, carbon black, and latex particles) were established by flow cytometry. DPM also induced a time-dependent increase in interleukin-8, granulocyte-macrophage colony-stimulating factor, and interleukin-1β release. The inflammatory response occurred later than phagocytosis and, because carbon black had no effect on cytokine release, its extent appeared to depend on the content of absorbed organic compounds. Furthermore, treatment of the exhaust gas to decrease the adsorbed organic fraction reduced the DPM-induced increase in granulocyte-macrophage colony-stimulating factor release. These results indicate that DPM can be phagocytosed by and induce a specific inflammatory response in airway epithelial cells.

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

potential to influence the development of airway disease through its adjuvant properties and by causing the release of proinflammatory mediators.

5.1.1.2. *Long-Term Exposures*

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

Battigelli et al. (1964) measured several indices of pulmonary function, including vital capacity, FEV_1, peak flow, nitrogen washout, and diffusion capacity in 210 locomotive repairmen exposed to diesel exhaust in 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 diesel exhaust showed marked seasonal variations because the doors of the engine house were open in the summer and closed in the winter. For the exposed group, the maximum daily workplace concentrations of air pollutants measured were 1.8 ppm NO_2, 1.7 ppm total aldehydes, 0.15 ppm acrolein, 4.0 ppm SO_2, and 5.0 ppm total hydrocarbons. The concentration of airborne particles was not reported.

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

Purdham et al. (1987) evaluated respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations who were exposed to both diesel and gasoline exhausts and in a control group of 11 on-site office workers. Twenty-four percent of the exposed group and 36% of the controls were smokers. If a particular symptom was considered to be influenced by smoking, smoking status was used as a covariate in the logistic regression analysis;

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

Additional epidemiological studies on the health hazards posed by exposure to diesel exhaust have been conducted for mining operations. Reger et al. (1982) evaluated the respiratory health status of 823 male coal miners from six diesel-equipped mines compared with 823 matched coal miners not exposed to diesel exhaust. The average tenure of underground work for the underground miners and their controls was only about 5 years; on average, the underground workers in diesel mines spent only 3 of those 5 years underground in diesel-use mines. Underground miners exposed to diesel exhaust reported a higher incidence of symptoms of cough and phlegm but proportionally fewer symptoms of moderate to severe dyspnea than their matched counterparts. These differences in prevalence of symptoms were not statistically significant. The diesel-exposed underground miners, on the average, had lower FVC, FEV_1, FEF_{50}, FEF_{75}, and FEF_{90} but higher peak flow and FEF_{25} than their matched controls. These differences, however, were not statistically significant. Health indicators for surface workers and their matched controls were directionally the same as for matched underground workers. There were no consistent relationships between the findings of increased respiratory symptoms, decreased pulmonary function, smoking history, years of exposure, or monitored atmosphere pollutants (NO_x, CO, particles, and aldehydes). Mean concentrations of NO_x at the six mines ranged from 0 to 0.6 ppm for short-term area samples, 0.13 to 0.28 ppm for full-shift personal

samples, and 0.03 to 0.80 for full-shift area samples. Inhalable particles (less than 10 μm MMAD) averaged 0.93 to 2.73 mg/m^3 for personal samples and 0 to 16.1 mg/m^3 for full shift area samples. Ames et al. (1984), using a portion of the miners studied by Reger, examined 280 diesel-exposed underground miners 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.

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

5.1.2. Laboratory Animal Studies

Because 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

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

Study	Description	Findings
	Acute exposures	
Kahn et al. (1988)	13 cases of acute exposure, Utah and Colorado coal miners.	Acute reversible sensory irritation, headache, nervous system effects, bronchoconstriction were reported at unknown exposures.
El Batawi and Noweir (1966)	161 workers, two diesel bus garages.	Eye irritation (42%), headache (37%), dizziness (30%), throat irritation (19%), and cough and phlegm (11%) were reported in this order of incidence by workers exposed in the service and repair of diesel-powered buses.
Battigelli (1965)	Six subjects, eye exposure chamber, three dilutions.	Time to onset was inversely related and severity of eye irritation was associated with the level of exposure to diesel exhaust.
Katz et al. (1960)	14 persons monitoring diesel exhaust in a train tunnel.	Three occasions of minor eye and throat irritation; no correlation established with concentrations of diesel exhaust components.
Hare and Springer (1971) Hare et al. (1974)	Volunteer panelists who evaluated general public's response to odor of diesel exhaust.	Slight odor intensity, 90% perceived, 60% objected; slight to moderate odor intensity, 95% perceived, 75% objected; moderate odor intensity, 100% perceived, almost 95% objected.
Linnell and Scott (1962)	Odor panel under highly controlled conditions determined odor threshold for diesel exhaust.	In six panelists, the volume of air required to dilute raw diesel exhaust to an odor threshold ranged from a factor of 140 to 475.
Rudell et al. (1990, 1994)	Eight healthy non-smoking subjects exposed for 60 min in chamber to diesel exhaust (3.7 ppm NO, 1.5 ppm NO_2, 27 ppm CO, 0.5 mg/m^3 formaldehyde, particles (4.3 × 10^6/cm^3). Exercise, 10 of each 20 min (75 W).	Odor, eye and nasal irritation in 5/8 subjects. BAL findings small decrease in mast cells, lymphocyte subsets and macrophage phagocytosis, small increase in PMNs.
Rudell et al. (1996)	Volunteers exposed to diesel exhaust for 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 concentration by trapping did not affect results.
Battigelli (1965)	13 volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 h.	No significant effects on pulmonary resistance were observed as measured by plethysmography.
Wade and Newman (1993)	Three railroad workers acutely exposed to diesel exhaust.	The workers developed symptoms of asthma.
Diaz-Sanchez et al. (1994)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	Enhancement of IgE production reported due to a dramatic increase in IgE-secreting cells.

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

Study	Description	Findings
Takenaka et al. (1995)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	DPM extracts enhanced interleukin-4 plus monoclonal antibody-stimulated IgE production as much as 360%, suggesting an enhancement of ongoing IgE production rather than inducing germline transcription or isotype switching.
Diaz-Sanchez et al. (1996)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	A broad increase in cytokine expression predicted to contribute to enhanced local IgE production.
Diaz-Sanchez et al. (1997)	Ragweed-sensitive volunteers challenged by a nasal spray of 0.30 mg DPM alone or in combination with ragweed allergen.	Ragweed allergen plus DPM-stimulated ragweed-specific IgE to a much greater degree than ragweed alone, suggesting DPM may be a key feature in stimulating allergen-induced respiratory allergic disease.
	Studies of cross-shift changes	
Reger (1979)	Five or more VC maneuvers by each of 60 coal miners exposed to diesel exhaust at the beginning and end of a workshift.	FEV_1, FVC, and PEFR were similar between diesel and non-diesel-exposed miners. Smokers had an increased number of decrements over shift than nonsmokers.
Ames et al. (1982)	Pulmonary function of 60 diesel-exposed compared with 90 non-diesel-exposed coal miners over workshift.	Significant workshift decrements occurred in miners in both groups who smoked; no significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed.
Jörgensen and Svensson (1970)	240 iron ore miners matched for diesel exposure, smoking, and age were given bronchitis questionnaires and spirometry pre- and postworkshift.	Among underground (surrogate for diesel exposure) miners, smokers, and older age groups, frequency of bronchitis was higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age.
Gamble et al. (1979)	200 salt miners performed before and after workshift spirometry. Personal environmental NO_2 and inhalable particle samples were collected.	Smokers had greater but not significant reductions in spirometry than ex- or nonsmokers. NO_2 but not particulate levels significantly decreased FEV1, FEF_{25}, FEF_{50}, and FEF_{75} over the workshift.
Gamble et al. (1987a)	232 workers in 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 14 years or more of induction latency time.
Edling et al. (1987)	Cohort of 694 male bus garage employees followed from 1951 through 1983 was evaluated for mortality from cardiovascular disease. Subcohorts categorized by levels of exposure were clerks (0), bus drivers (1), and bus garage employees (2).	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as subcohorts with different levels of exposure.

and lung damage was observed in the exposed animals. The exhaust contained 74 mg/m^3 DPM (particle size not reported), 560 ppm CO, 23 ppm NO_2, and 16 ppm aldehydes. Exhaust containing 5 mg/m^3 DPM, 380 ppm CO, 43 ppm NO_2, and 6.4 ppm aldehydes resulted in low mortality rates (mostly below 10%) and moderate lung damage. Exhaust containing 122 mg/m^3 DPM, 418 ppm CO, 51 ppm NO_2, and 6.0 ppm aldehydes produced high mortality rates (mostly above 50%) and severe lung damage. Exhaust containing 1,070 mg/m^3 DPM, 1,700 ppm CO, 12 ppm NO_2, and 154 ppm aldehydes resulted in 100% mortality in all three species. High CO levels, which resulted in a carboxyhemoglobin value of 60% in mice and 50% in rabbits and guinea pigs, were considered to be the main cause of death in the latter case. High NO_2 levels were considered to be the main cause of lung damage and mortality seen in the other three tests. Aldehydes and NO_2 were considered to be the main irritants in the light load test.

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

The effects of DPM and its components (extracted particles and particle extracts) on the release of proinflammatory cytokines, interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α)

by alveolar macrophages (AMs) were investigated by Yang et al. (1997). Rat AMs 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 concentrations, both DPM and DPM extracts were shown to increase IL-1-like activity secreted by AMs, whereas extracted particles had no effect. Neither particles, particle extracts, or extracted particles stimulated secretion of TNF-α. DPM inhibited lipid polysaccharide (LPS)-stimulated production of IL-1 and TNF-α. In contrast, interferon- (IFN) γ stimulated production of TNF-α was not affected by DPM. Results of this study indicate that the organic fraction of exhaust particles is responsible for the effects noted. Stimulation of IL-1 but not TNF-α suggests that IL-1, but not TNF-α, 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-α by LPS is unknown, but may be a contributing factor to the decreased AM phagocytic activity and increased susceptibility to pulmonary infection after prolonged exposure to DPM.

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

5.1.2.2. *Short-Term and Subchronic Exposures*

A number of inhalation studies have employed a regimen of 20 h/day, 7 days/week for varying exposure periods up to 20 weeks to differing concentrations of airborne particulate matter, vapor, and gas concentrations of diluted diesel exhaust. Exposure regimens and characterization of gas-phase components for these studies are summarized in Table 5-2.

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

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

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

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

[a]Irradiated exhaust.

PMN = Polymorphonuclear leukocyte.

AM = Alveolar macrophage.

Pepelko et al. (1980a) evaluated the pulmonary function of cats exposed under these conditions for 28 days to 6.4 mg/m^3 DPM. The only significant functional change observed was a decrease in maximum expiratory flow rate at 10% vital capacity. The excised lungs of the exposed cats appeared charcoal gray, with focal black spots visible on the pleural surface. Pathologic changes included a predominantly peribronchial localization of black-pigmented macrophages within the alveoli characteristic of focal pneumonitis or alveolitis.

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

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

A separate group of adult guinea pigs was necropsied after 56 days of exposure to IE, to diluted RE, or to clean air (Wiester et al., 1980). Exposure resulted in a significant increase in the ratio of lung weight to body weight (0.68% for controls, 0.78% for IE, and 0.82% for RE). Heart/body weight ratios were not affected by exposure. Microscopically, there was a marked accumulation of black pigment-laden 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 DPM. Two rats from the exposed group and one

rat from the control group (filtered room air) were sacrificed after each exposure interval of 6 h and 1, 3, 7, 14, 28, 42, and 63 days; daily exposures were for 20 h and were 5.5 days/week. Evidence of AM recruitment and phagocytosis of diesel particles was found at the 6-h sacrifice; after 24 h of exposure there was a focal, scattered increase in the number of Type II cells. After 4 weeks of exposure, there were morphologic changes in size, content, and shape of AM, septal thickening adjacent to clusters of AMs, and an appearance of inflammatory cells, primarily within the septa. At 9 weeks of exposure, focal aggregations of particle-laden macrophages developed near the terminal bronchi, along with an influx of polymorphonuclear Leukocytes (PMNS), Type II cell proliferation, and thickening of alveolar walls. The affected alveoli occurred in clusters that, for the most part, were located near the terminal bronchioles, but occasionally were focally located in the lung parenchyma. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

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

Kaplan et al. (1982) evaluated the effects of subchronic exposure to diesel exhaust on rats, hamsters, and mice. The exhaust was diluted to a concentration of 1.5 mg/m^3 DPM; exposures were 20 h/day, 7 days/week. Hamsters were exposed for 86 days, rats and mice for 90 days. There were no significant differences in mortality or growth rates between exposed and control animals. Lung weight relative to body weight of rats exposed for 90 days was significantly higher than the mean for the control group. Histological examination of tissues of all three species indicated particle accumulation in the lungs and mediastinal lymph nodes. Associated with the larger accumulations, there was a minimal increase in the thickness of the

alveolar walls, but the vast majority of the particles elicited no response. After 6 mo of recovery, considerable clearance of the DPM from the lungs occurred in all three species, as evaluated by gross pathology and histopathology. However, no quantitative estimate of clearance was provided.

Toxic effects in animals from acute exposure to diesel exhaust appear to be primarily attributable to the gaseous components (i.e., mortality from CO intoxication and lung injury caused by cellular damage resulting from NO_2 exposure). The results from short-term exposures indicate that rats experience minimal lung function impairment even at diesel exhaust levels sufficiently high to cause histological and cytological changes in the lung. In subchronic studies of durations of 4 weeks or more, frank adverse health effects are not readily apparent and, when found, are mild and result from exposure to concentrations of about 6 mg/m^3 DPM and durations of exposures of 20 h/day. There is ample evidence that subchronic exposure to lower levels of diesel exhaust affects the lung, as indicated by accumulation of particles, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and thickening of alveolar walls adjacent to AM aggregates. Little evidence exists, however, that subchronic exposure to diesel exhaust impairs lung function. Recent studies have implicated the organic fraction of DPM in the induction of respiratory allergic disease.

5.1.2.3. *Chronic Exposures*

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

Increased lung weights and lung-to-body weight ratios have been reported in rats, mice, and hamsters. These data are summarized in Table 5-4. In rats exposed for up to 36 weeks to 0.25 or 1.5 mg/m^3 DPM, lung wet weights (normalized to body weight) were significantly higher in the 1.5 mg/m^3 exposure group than control values after 12 weeks of exposure (Misiorowski et al., 1980). Rats and Syrian hamsters were exposed for 2 years (five 16-h periods per week) to diesel exhaust diluted to achieve concentrations of 0.7, 2.2, and 6.6 mg/m^3 DPM (Brightwell et

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

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

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

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

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

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	14.2	2.3	2.8	Increased lung weight	Heinrich et al. (1995)
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Increase in lung weight was significant at 2 and 6 mg/m^3	Nikula et al. (1995)
Rat		0.8 2.5 6.98					Increased lung weight in rats and mice at 3.5 and 7 mg/m^3	Henderson et al. (1988)
Mouse		6.98 4.5						

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

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

Heinrich et al. (1995) reported that rats exposed to 2.5 and 7 mg/m^3 DPM for 18 h/day, 5 days/week for 24 mo showed significantly lower body weights than 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 insignificant decreases in body weight. Lung weights were dramatically affected, with increases progressing throughout the study from 1.5-fold at 3 mo to 3-fold at 12 mo. Mice (NMRI and C57BL/6N strains) were also exposed to 4.5 mg/m^3 for 23 mo. In NMRI mice, the body weights were reported to be significantly lower than controls, but the magnitude of the change is not reported so biological significance cannot be assessed. Mortality was slightly increased, but statistical significance is not reported. The C57BL/6N mice showed minimal effects on body weight and mortality, which were not statistically significant. Lung weights were dramatically affected in both strains.

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

Significant increases in lung weight were first seen at 6 mo in the high-exposure group and at 12 to 18 mo in the low-exposure group.

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

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

Lewis et al. (1989) evaluated functional residual capacity and airway resistance and conductance in 10 control and 10 diesel-exposed rats (2 mg/m^3 DPM, 7 h/day, 5 days/week for 52 or 104 weeks). At the 104-week evaluation, the rats were also examined for maximum flow volume impairments. No evidence of impaired pulmonary function as a result of the exposure to diesel exhaust was found in rats. Lewis et al. (1989) exposed male cynomolgus monkeys to diesel exhaust for 7 h/day, 5 days/week, for 24 mo. Groups of 15 monkeys were exposed to air, diesel exhaust (2 mg/m^3), coal dust, or combined coal dust and diesel exhaust. Pulmonary function was evaluated prior to exposure and at 6-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 decreased. The measurement of forced expiratory flow at 40% of total lung capacity was significantly decreased

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 7.1 0.23-0.26 μm MMD	1,593 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Diffusing capacity, lung compliance reduced at 3.5 and 7 mg/m^3	Mauderly et al. (1988) McClellan et al. (1986)
Rat, F344, M, F; Hamster Syrian, M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — —	— — —	— — —	Large number of pulmonary function changes consistent with obstructive and restrictive airway diseases at 6.6 mg/m^3 (no specific data provided)	Brightwell et al. (1986)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4.24 0.35 μm MMD	48,336	12.5	1.5	1.1	Significant increase in airway resistance	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24 0.35 μm MMD	56,392	12.5	1.5	1.1	Decrease in dynamic lung compliance; increase in airway resistance	Heinrich et al. (1986a)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0[a] 12.0[b]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	Decrease in vital capacity, total lung capacity, and diffusing capacity after 2 years; no effect on expiratory flow	Pepelko et al. (1980b, 1981) Moorman et al. (1985)

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

at 12, 18, and 24 mo of exposure. The finding of an obstructive effect in monkeys contrasts with the finding of restrictive type effects in other laboratory animal species (Vinegar et al., 1980, 1981a; Mauderly et al., 1988; Pepelko et al., 1980b, 1981) and suggests a possible difference in effect between primate and small animal respiratory tracts. In these monkeys there were no specific histopathological effects reported (see next section) although particle aggregates were reported in the distal airways, suggesting more small airway deposition.

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

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

Hamsters (eight or nine per group) were exposed 8 h/day, 7 days/week, for 6 mo to concentrations of either about 6 mg/m^3 or about 12 mg/m^3 DPM (Vinegar et al., 1980, 1981a,b). Vital capacity, vital capacity/lung weight ratio, residual lung volume by water displacement, and CO_2 diffusing capacity decreased significantly in hamsters exposed to 6 mg/m^3 DPM. Static deflation volume-pressure curves showed depressed deflation volumes for diesel-exposed hamsters when volumes were corrected for body weight and even greater depressed volumes when volumes were corrected for lung weight. However, when volumes were expressed as percentage of vital capacity, the diesel-exposed hamsters had higher lung volumes at 0 and 5 cm H_2O. In the absence of confirmatory histopathology, the authors tentatively concluded that these elevated lung volumes and the significantly reduced diffusing capacity in the same hamsters

were indicative of possible emphysematous changes in the lung. Similar lung function changes were reported in hamsters exposed at 12 mg/m^3 DPM, but detailed information was not reported. It was stated, however, that the decrease in vital capacity was 176% greater in the second experiment than in the first.

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

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

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

Pepelko et al. (1980b; 1981; see also Pepelko, 1982b) and Moorman et al. (1985) measured the lung function of adult cats chronically exposed to diesel exhaust. The cats were exposed for 8 h/day and 7 days/week for 124 weeks. Exposures were at 6 mg/m^3 for the first 61 weeks and 12 mg/m^3 from weeks 62 to 124. No definitive pattern of pulmonary function

changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes (TLC, FVC, FRC, and inspiratory capacity [IC]) and the significantly lower single-breath diffusing capacity, coupled with normal values for dynamic ventilatory function (mechanics of breathing), indicate the presence of a lesion that restricts inspiration but does not cause airway obstruction or loss of elasticity. This pulmonary physiological syndrome is consistent with an interstitial fibrotic response that was later verified by histopathology (Plopper et al., 1983).

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

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

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

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; bronchiolo-alveolar hyperplasia; alveolar lipo-proteinosis; 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)
Mouse, CD-1, M,F	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 μm MDD	1,274 12,740 25,844	3 17 30	0.1 0.3 0.7	— — —	Exposure-related increase in lung soot, pigment-laden macrophages, lung lesions. Bronchiolization in alveolar ducts at 7.1 mg/m^3	Mauderly et al. (1996)

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

AM = Alveolar macrophage.
PMN = Polymorphonuclear leukocyte.

ducts. Associated with these masses was a minimal increase in the thickness of the alveolar walls; however, the vast majority of the particles elicited no response. After 6 mo of recovery, the lungs of all three species contained considerably less pigment, as assessed by gross pathological and histopathological examinations.

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

Nikula et al. (1997) reevaluated the lung tissue from this study. They concluded that there were no significant differences in the amount of retained particulate matter between monkeys and rats exposed under the same conditions. The rats, however, retained a greater portion of the particulate matter in lumens of the alveolar ducts and alveoli than did the monkeys. Conversely, monkeys retained a greater portion of the particulate material in the interstitium than did rats. Aggregations of particle laden macrophages in the alveoli were rare, and there were few signs of particle-associated inflammation in the monkeys. Minimal histopathologic lesions were detected in the interstitium. 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 DPM. Rats were exposed for 16 h/day, 6 days/week for 30 mo. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 DPM or less. At concentrations above 0.4 mg/m^3 DPM, severe morphological changes were observed. These changes consisted of shortened and absent cilia in the tracheal and bronchial epithelium, marked hyperplasia of the bronchiolar epithelium, and swelling of the Type II cellular epithelium. These lesions appeared to increase in severity with increases in exhaust concentration and duration of exposure. There was no difference in the degree of changes in pulmonary pathology at the same concentrations between the LD and the HD series.

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 DPM.

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 DPM. At 1 and 3 mo of exposure, there were minimal histological changes in the lungs of the exposed rats. After 6 mo of exposure, there were particle-laden macrophages distributed irregularly throughout the lung and a proliferation of Type II cells with adenomatous metaplasia in areas where the macrophages had accumulated. After 1 year of exposure, foci of heterotrophic hyperplasia of ciliated or nonciliated bronchiolar epithelium on the adjacent alveolar walls were more common, the quantity of deposited particulate matter increased, and the number of degenerative AMs and proliferative lesions of Type II or bronchiolar epithelial cells increased. After 2 years of exposure, there was a fibrous

thickening of the alveolar walls, mast cell infiltration with epithelial hyperplasia in areas where the macrophages had accumulated, and neoplasms.

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

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

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

Histological changes in the lungs of guinea pigs exposed to diluted diesel exhaust containing either 0.25, 0.75, 1.5, or 6.0 mg/m^3 DPM were reported by Barnhart et al. (1981; 1982). Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo resulted in an uptake of exhaust

particles by three alveolar cell types (AMs, Type I cells, and interstitial macrophages) and also by granulocytic leukocytes (eosinophils). The alveolar-capillary membrane increased in thickness as a result of an increase in the absolute tissue volume of interstitium and Type II cells. In a continuation of these studies, guinea pigs were exposed to diesel exhaust (up to 6.0 mg/m^3 DPM) for 2 years (Barnhart et al., 1982). A minimal tissue response occurred at 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 DPM were characterized by large numbers of black AMs in the alveolar spaces, thickening of the alveolar epithelium, hyperplasia of Type II cells, and edema.

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

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

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

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

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

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

were generally much greater in rats than in hamsters. Exposures were to diesel exhaust containing 4.24 mg/m^3 DPM for 19 h/day, 5 days/week for 120 (hamsters) to 140 (rats) weeks.

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

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

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

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

A number of reports (McClellan et al., 1986; Mauderly et al., 1987a, 1990a; Henderson et al., 1988) have addressed biochemical and cytological changes in lung tissue and BALF of rodents exposed for 7 h/day, 5 days/week for up to 30 mo at concentrations of 0, 0.35, 3.5, or 7.1 mg/m^3 DPM. At the lowest exposure level (0.35 mg/m^3), no biochemical or cytological changes occurred in the BALF or in lung tissue in either Fischer 344 rats or CD-1 mice. Henderson et al. (1988) provide considerable time-course information on inflammatory events taking place throughout a chronic exposure. A chronic inflammatory response was seen at the two higher exposure levels in both species, as evidenced by increases in inflammatory cells (macrophages and neutrophils), cytoplasmic and lysosomal enzymes (lactate dehydrogenase, glutathione reductase, and 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 DPM) 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 (Hyde et al., 1985). Exposures were for 7 h/day, 5 days/week at 6 mg/m^3 DPM for 61 weeks and at 12 mg/m^3 for weeks 62 to 124.

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

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

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

Several studies have been conducted investigating the effect of inhaled diesel exhaust on the deposition and fate of inert tracer particles or diesel particles themselves. Lung clearance of deposited particles occurs in two distinct phases: a rapid phase (hours to days) from the tracheobronchial region via the mucociliary escalator and a much slower phase (weeks to 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 DPM. The exposure to 17 mg/m^3 resulted in decreased clearance after a single exposure as well as after a cumulative exposure of 34 or

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)

[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.

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

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

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

Heinrich et al. (1982) evaluated lung clearance in rats exposed for approximately 18 mo at 3.9 mg/m^3 DPM for 7 to 8 h/day, 5 days/week. Following exposure to $^{59}Fe_2O_3$-aerosol, the rats were returned to the diesel exhaust exposure and the radioactivity was measured over the thoracic area at subsequent times. The biological half-life of the iron oxide deposited in the rats' lungs was nearly twice that of controls.

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

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

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

Mauderly et al. compared the effects of diesel exhaust in normal lungs with rats in which emphysema had been induced experimentally by instillation of elastase 6 weeks before diesel exhaust exposures. The rats were exposed to 3.5 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Measurements included histopathology, clearance, pulmonary function, lung lavage, and

immune response. In the rats that were not pretreated with elastase, there was a significant reduction in the number of macrophages recovered by pulmonary lavage in contrast to the increases in macrophages reported by Strom (1984) and Henderson et al. (1988). The half-time of the slow phase of clearance of inhaled, 1 μm, monodisperse particles was doubled in the exposure animals without elastase pretreatment. The elastase pretreatment did not affect clearance in unexposed animals but significantly reduced the effect of diesel. The clearance half-time was significantly less in elastase-pretreated, diesel-exposed animals than in diesel-exposed normal animals. Many other effects measured in this study were also less affected by diesel exposure in elastase-treated animals. Measurements of lung burden of DPM showed that elastase-pretreated animals accumulated less than half as much DPM mass as normal animals exposed at the same time, suggesting that the difference in effect could be explained by differences in dose to the lung.

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

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

Exposure to diesel exhaust has been reported both to increase the number of recoverable AMs from the lung (Strom, 1984; Vostal et al., 1982; Henderson et al., 1988) or to produce no change in numbers (Chen et al., 1980; Castranova et al., 1985). Strom (1984) found that in rats exposed to 0.25 mg/m^3 DPM for 20 h/day, 5.5 days/week for 6 mo or 1 year, as well as in the

controls, BAL cells consisted entirely of AMs, with no differences in the cell counts in the lavage fluid. At the higher concentrations, 0.75 or 1.5 mg DPM/m^3, the count of AM increased proportionally with the exposure concentration; the results were identical for AMs at both 6 and 11 or 12 mo of exposure. The increase in AM counts was much larger after exposure to 1.5 mg/m^3 DPM for 6 mo than after exposure to 0.75 mg/m^3 for 1 year, although the total mass (calculated as C × T) of deposited particulate burden was the same. These data suggested to the authors that the number of lavaged AMs was proportional to the mass influx of particles rather than to the actual DPM burden in the lung. These results further implied that there may be a threshold for the rate of mass influx of DPM into the lungs of rats above which there was an increased recruitment of AMs. Henderson et al. (1988) reported similar findings of significant increases of AMs in rats and mice exposed to 7.1 mg/m^3 DPM for 18 and 24 mo, respectively, for 7 h/day, 5 days/week, but not at concentrations of 3.5 or 0.35 mg/m^3 for the same exposure durations. Chen et al. (1980), using an exposure regimen of 0.25 and 1.5 mg/m^3 DPM for 2 mo and 20 h/day and 5.5 days/week, found no significant changes in absolute numbers of AMs from guinea pig bronchoalveolar lavage fluid (BALF), nor did Castranova et al. (1985) in rat BALF following exposure to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 2 years.

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

The integrity of pulmonary defense mechanisms can also be ascertained by assessing if exposure to diesel exhaust affects colonization and clearance of pathogens and alters the response of the challenged animals to respiratory tract infections. Campbell et al. (1980, 1981) exposed mice to diesel exhaust followed by infectious challenge with *Salmonella typhimurium*, *Streptococcus pyogenes*, or A/PR8-3 influenza virus and measured microbial-induced mortality.

Exposures to diesel exhaust were to 6 mg/m^3 DPM for 8 h/day, 7 days/week for up to 321 days. Exposure to diesel exhaust resulted in enhanced susceptibility to the lethal effects of *S. pyogenes* infection at all exposure durations (2 h, 6 h; 8, 15, 16, 307, and 321 days). Tests with *S. typhimurium* were inconclusive because of high mortality rates in the controls. Mice exposed to diesel exhaust did not exhibit an enhanced mortality when challenged with the influenza virus. Hatch et al. (1985) found no changes in the susceptibility of mice to Group C *Streptococcus* sp. infection following intratracheal injection of 100 μg of DPM suspended in unbuffered saline.

Hahon et al. (1985) assessed virus-induced mortality, virus multiplication with concomitant interferon (IFN) levels (lungs and sera), antibody response, and lung histopathology in mice exposed to diesel exhaust prior to infectious challenge with Ao/PR/8/34 influenza virus. Weanling mice were exposed to 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—inhalation studies.*** The effects of diesel exhaust on the immune system of guinea pigs were investigated by Dziedzic (1981). Exposures were to 1.5 mg/m^3 DPM for 20 h/day, 5.5 days/week for up to 8 weeks. There was no effect of diesel

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

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

Bice et al. (1985) evaluated whether or not exposure to diesel exhaust would alter antibody immune responses induced after lung immunization of rats and mice. Exposures were to 0.35, 3.5, or 7.1 mg/m^3 DPM for 7 h/day, 5 days/week for 24 mo. Chamber controls and exposed animals were immunized by intratracheal instillation of 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.

The effects of inhaled diesel exhaust and DPM have been studied in a murine model of allergic asthma (Takano et al., 1998a,b). ICR mice were exposed for 12 h/day, 7days/week for 40 weeks to diesel exhaust (0.3, 1.0, or 3.0 mg/m^3). The mice were sensitized with ovalbumin (OA) after 16 weeks exposure and subsequently challenged with aerosol allergen (1% OA in isotonic saline for 6 min) at 3-week intervals during the last 24 weeks of exposure. Exposure to diesel exhaust enhanced allergen-related eosinophil recruitment to the submucosal layers of the airways and to the bronchoalveolar space, and increased protein levels of granulocyte-colony

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

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

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

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

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

stimulating factor (GM-CSF) and IL-5 in the lung in a dose-dependent manner. In the diesel exhaust-exposed mice, increases in eosinophil recruitment and local cytokine expression were accompanied by goblet cell proliferation in the bronchial epithelium and airway hyperresponsiveness to inhaled acetylcholine. In contrast, mice exposed to clean air or diesel exhaust without allergen provocation showed no eosinophil recruitment to the submucosal layers of the airways or to the bronchoalveolar space, and few goblet cells in the bronchial epithelium. The authors concluded that daily inhalation of diesel exhaust can enhance allergen-related respiratory diseases such as allergic asthma and this effect may be mediated by the enhanced local expression of IL-5 and GM-CSF. The effects of DPM on a second characteristic of allergic asthma, airway hyperresponsiveness, was examined by Takano et al. (1998b). Laboratory mice were administered OA, DPM, or OA and DPM combined by intratracheal instillation for 6 wk. Respiratory resistance (Rrs) after acetylcholine challenge was measured 24 h after the final instillation. Rrs was significantly greater in the mice treated with OA and DPM than in the other treatments. The authors concluded that DPM can enhance airway responsiveness associated with allergen exposure.

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

diesel exhaust exposure alone. These workers concluded that inhalation of diesel exhaust can enhance airway hyperresponsiveness and airway inflammation caused by OA sensitization in mice.

The effects of diesel exhaust on IgE antibody production were investigated in BALB/c mice sensitized with OA and exposed by inhalation to diesel exhaust (3.0 and 6.0 mg/m^3) for 3 weeks (Fujimaki et al., 1997). The mice were sensitized by intranasal administration of OA alone before, immediately after, and 3 weeks after diesel exhaust inhalation. While body and thymus weights were unchanged in the diesel exhaust-exposed and control mice, spleen weights in mice exposed to 6 mg/m^3 diesel exhaust increased significantly. Anti-OA IgE antibody titers in the sera of mice exposed to 6 mg/m^3 diesel exhaust were significantly higher than control. Total IgE and anti-OA IgG in sera from diesel exhaust-exposed and control mice remained unchanged. Cytokine production was measured in vitro stimulated with OA in spleen cells from mice exposed to diesel exhaust (6 mg/m^3). Antigen-stimulated interleukin-4 (IL-4) and -10 (IL-10) production increased significantly in vitro in spleen cells from diesel exhaust-exposed mice compared to control, while interferon (IFN)-g production decreased markedly. The authors concluded that diesel exhaust-inhalation in mice may affect antigen-specific IgE antibody production through alteration of the cytokine network.

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

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

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

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

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

Model	Treatment	Effects	Reference
Mouse, ICR, M	Intratracheal instillation of OA, DPM, or OVA and DPM combined, once/week for 6 wk.	Respiratory resistance (Rrs) measured 24 h after the final instillation. Rrs after acetylcholine challenge was significantly greater in the mice treated with OVA and DPM than other treatments. DPM can enhance airway responsiveness associated with allergen exposure.	Takano et al. (1998b)

OA- Ovalbumin
DPM- diesel particulate matter
CB- carbon black

PEG-SOD- polyethyleneglycol-conjugated superoxide dismutase
IL-4-interleukin-4
IL-5- interleukin-5
IL-10- interleukin-10
IFN- interferon-g
GM-CSF -granulocyte-colony stimulating factor
IP-intraperitoneally

Respiratory resistance to acetylcholine in the DPM-group was 11 times higher than in controls, and the increased resistance was significantly suppressed by PEG-SOD pretreatment. These findings indicate that oxygen radicals, caused by intratracheally instilled DPM elicits responses characteristic of bronchial asthma.

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

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

Maejima et al. (1997) examined the potential adjuvant activity of several different fine particles. These workers administered 25 μg of each of 5 particles (Kanto loam dust, fly ash, carbon black, DPM, and aluminum hydroxide [alum]) intranasally in mice and exposed them to aerosolized Japanese cedar pollen allergens (JCPA) for intervals up to 18 weeks. Measurements were made of JCPA-specific IgE and IgG antibody titers, the protein-adsorbing capacity of each type of particle, and nasal rubbing movements (a parameter of allergic rhinitis in mice). The increases in anti-JPCA IgE and IgG antibody titers were significantly greater in mice treated with particles and aerosolized JCPA than in mice treated with aerosolized JCPA alone. In a

subsequent experiment, the mice received the particles as before, but about 160,000 grains of Japanese cedar pollen (JCP) were dropped onto the tip of the nose of each mouse twice a week for 16 wk. After 18 wk there were no significant differences in the anti-JCPA IgE and IgG production, nasal rubbing, or histopathological changes. The workers concluded that the nature of the particle, the ability of the particle to absorb antigens, and/or particle size is not related to the enhancement of IgE antibody production or symptoms of allergic rhinitis. However, IgE antibody production did appear to occur earlier in mice treated with particles than in mice immunized with allergens alone.

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

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

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

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

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

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

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

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

[a]1 to 61 weeks of exposure.

[b]62 to 124 weeks of exposure.

5.1.2.3.8. ***Blood and cardiovascular systems.*** Several studies have evaluated the effects of diesel exhaust exposure on hematological and cardiovascular parameters of laboratory animals. These studies are summarized in Table 5-11. Standard hematological indices of toxicological effects on red and white blood cells failed to detect dramatic and consistent responses. Erythrocyte (RBC) counts were reported as being unaffected in cats (Pepelko and Peirano, 1983), rats and monkeys (Lewis et al., 1989), guinea pigs and rats (Penney et al., 1981), and rats (Karagianes et al., 1981); lowered in rats (Heinrich et al., 1982); and elevated in rats (Research Committee for HERP Studies, 1988; Brightwell et al., 1986). Mean corpuscular volume was 0 significantly increased in monkeys, 69 versus 64 (Lewis et al., 1989), and hamsters (Heinrich et al., 1982) and lowered in rats (Research Committee for HERP Studies, 1988). The only other parameters of erythrocyte status and related events were lowered mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in rats (Research Committee for HERP Studies, 1988), a 3% to 5% increase in carboxyhemoglobin saturation in rats (Karagianes et al., 1981), and a suggestion of an increase in prothrombin time (Brightwell et al., 1986). The biological significance of these findings regarding adverse health effects is deemed to be inconsequential.

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

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 hypoxia. 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.

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

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

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

Key: MCV = Mean corpuscular volume.

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

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

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

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

Clinical chemistry studies suggest impairment of both liver and kidney functions in rats and hamsters chronically exposed to high concentrations of diesel exhaust. The absence of histopathological confirmation, the appearance of such effects near the end of the lifespan of the laboratory animal, and the failure to find such biochemical changes in cats exposed to a higher

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

Species/sex	Exposure period	Particles (mg/m³)	C × T (mg·h/m³)	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/m³, 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/m³, decrease in potassium, LDH, aspartate amino-transferase; increase in albumin and gamma-glutamyl transferase	Brightwell et al. (1986)
Cat inbred, M	8 h/day 7 days/week 124 weeks	6.0[c] 12.0[d]	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	BUN unaltered; SGOT and SGPT unaffected; LHD increase after 1 year of exposure	Pepelko and Peirano (1983)

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

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

dose, however, tend to discredit the probability of hepatic and renal hazards to humans exposed at atmospheric levels of diesel exhaust.

5.1.2.3.10. ***Effects on microsomal enzymes.*** Several studies have examined the effects of diesel exhaust exposure on microsomal enzymes associated with the metabolism and possible activation of xenobiotics, especially polynuclear aromatic hydrocarbons. These studies are summarized in Table 5-13. Lee et al. (1980) measured the activities of aryl hydrocarbon hydroxylase (AHH) and epoxide hydrase (EH) in liver, lung, testis, and prostate gland of adult male rats exposed to 6.32 mg/m^3 DPM 20 h/day for 42 days. Maximal significant AHH activities (pmol/min/mg microsomal protein) occurred at different times during the exposure period, and differences between controls and exposed rats, respectively, were as follows: prostate 0.29 versus 1.31, lung 3.67 versus 5.11, and liver 113.9 versus 164.0. There was no difference in AHH activity in the testis between exposed and control rats. Epoxide hydrase activity was not significantly different from control values for any of the organs tested.

Pepelko and Peirano (1983) found no 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 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 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 nonmicrosomal lung protein present in the microsomal preparations. Exposures to 6 mg/m^3 DPM were for 8 h/day, 7 days/week.

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

Species/sex	Exposure period	Particles (mg/m^3)	C × t ($mg \cdot h/m^3$)	Co (ppm)	No_2 (ppm)	So_2 (ppm)	Effects	Study
Rat, f344, m	—	—	—	—	—	—	Intratracheal administration of dpm extract required doses greater than 6 mg/m^3 before the lung ahh was barely doubled; liver ahh activity was unchanged	Chen (1986)
Mouse, cd-1, f	7 h/day 5 days/week 4 weeks	2.0 0.2-0.36 μm mdd	280	11.5	1.5	0.8	Mice inoculated intranasally with influenza virus had smaller increases in ethylmorphine demethylase activity on days 2 to 4 postvirus infection and abolition of day 4 postinfection increase in nadph-dependent cytochrome c reductase	Rabovsky et al. (1986)
Rat, sprague-dawley, m	20 h/day 7 days/week 1-7 weeks	6.3	882-6,174	17.4	2.3	2.1	Ahh induction occurred in lung, liver, and prostate gland but not in testes; maximum significant activities occurred at different times; liver has greatest overall activity, percent increase highest in prostate; expoxide hydrase activity was unaffected	Lee et al. (1980)
Rat, f344, m	20 h/day 5.5 days/week 4, 13, 26, or 39 weeks	0.75 1.5 0.19 μm mdd	330-6,435	4.8 7.5	— —	— —	Inhalation exposure had no significant effect on liver ahh activity; lung ahh activity was slightly reduced after 6-mo exposure to 1.5 mg/m^3 dpm; an ip dose of dp extract, estimated to be equivalent to inhalation exposure, had no effect on ahh activity in liver and lungs; cyt. P-50 was unchanged in lungs and liver following inhalation or ip administration	Chen and vostal (1981)
	20 h/day 5.5 days/week 4, 13, 26, or 39 weeks	0.75 1.5 0.19 μm mdd	330-6,435	4.8 7.5	— —	— —		
Rat, f344, f	7 h/day 5 days/week 12, 26, or 104 weeks	2.0 0.23-0.36 μm mdd	840-7,280	11.5	1.5	0.8	No effect on b[*a*]p hydrolase or 7-exthoxycoumarin deethylase activities in the liver	Rabovsky et al. (1984)
Rat, f344, m	20 h/day 5.5 days/week 8-53 weeks	0.25 1.5 0.19 μm mdd	220-8,745	2.9 7.5	— —	— —	After 8 weeks, no induction of cyt. P-450, cyt. P-448, or nadph-dependent cyt. c reductase; after 1 year of exposure, liver microsomal oxidation of b[*a*]p was not increased; 1 year of exposure to either 0.25 or 1.5 mg/m^3 dpm impaired lung microsomal metabolism of b[*a*]p	Navarro et al. (1981)
Mouse, a/j, m	8 days/week 7 days/week 26 or 35 weeks	6.0	17.4	17.4	2.3	2.1	No differences in lung and liver ahh activities and liver p-448, p-450 levels	Pepelko and Peirano (1983)

Ahh = aryl hydrocarbon hydroclase.
B[*a*]p = benzo[*a*]pyrene.

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.

5.1.2.3.11. ***Effects on behavior and neurophysiology.*** Studies on the effects of exposure to diesel exhaust on the behavior and neurophysiology of laboratory animals are summarized in Table 5-14. Laurie et al. (1978) and Laurie et al. (1980) examined behavioral alterations in adult and neonatal rats exposed to diesel exhaust. Exposure for 20 h/day, 7 days/week, for 6 weeks to exhaust containing 6 mg/m^3 DPM produced a significant reduction in adult spontaneous locomotor activity (SLA) and in neonatal pivoting (Laurie et al., 1978). In a follow-up study, Laurie et al. (1980) found that shorter exposure (8 h/day) to 6 mg/m^3 DPM also resulted in a reduction of SLA in adult rats. Laurie et al. (1980) conducted additional behavioral tests on adult rats exposed during their neonatal period. For two of three exposure situations (20 h/day for 17 days postparturition, or 8 h/day for the first 28 or 42 days postparturition), significantly lower SLA was observed in the majority of the tests conducted on the adults after week 5 of measurement. When compared with control rats, adult 15-month-old rats that had been exposed as neonates (20 h/day for 17 days) also exhibited a significantly slower rate of acquisition of a bar-pressing task to obtain food. The investigators noted that the evidence was insufficient to determine whether the differences were the result of a learning deficit or due to some other cause (e.g., motivational or arousal differences).

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

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

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

SLA = Spontaneous locomotor activity.

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.

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

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

Several studies have evaluated the effect of exposure to diesel exhaust on sperm. Lewis et al. (1989) found no adverse sperm effects (sperm motility, velocity, densities, morphology, or incidence of abnormal sperm) in monkeys exposed for 7 h/day, 5 days/week, for 104 weeks to 2mg/m^3 DPM. In another study in which A/Strong mice were exposed to diesel exhaust containing

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

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

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.,). It was noted, however, that there was a high rate of spontaneous sperm abnormalities in this strain of mice, and this may have masked any small positive effect. Quinto and De Marinis (1984) reported a statistically significant and dose-related increase in sperm abnormalities in mice injected intraperitoneally for 5 days with 50, 100, or 200 mg/kg of DPM suspended in corn oil. A significant decrease in sperm number was seen at the highest dose, but testicular weight was unaffected by the treatment.

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

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

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

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

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

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

Species/sex	Exposure[b] period		Particles (mg/m^3)	C × t ($mg \cdot h/m^3$)	Co (ppm)	No_2 (ppm)	So_2 (ppm)	Effects	Study
Rat wistar, f;	7 h/day	Uf	3.9	14,196	18.5	1.2	3.1	No effect on pulmonary function or heart rate in rats; increases in	Heinrich et al.
hamster, syrian	5 days/week	F	—		18.0	1.0	2.8	pulmonary adenomatous prolifcrations in hamsters, uf	(1982)
	104 weeks	C	—		—	—	—	significantly higher than f or c	
Rat, f344, f	8 h/day	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	Iwai et al. (1986)
	7 days/week	F[c]	—		—	—	—	rate weight rate higher in both groups at 24 mo; at 2 years,	
	104 weeks	C	—		—	—	—	fibrosis and epithelial hyperplasia in lungs of uf; nominal lung and spleen histologic changes	
Rat, f344, m, f;	16 h/day	Uf	0.7	5,824	—	—	—	Uf: elevated red and white cell counts, hematocrit and hemo-	Brightwell et al.
hamster, syrian,	5 days/week	Uf	2.2	18,304	—	—	—	globin; increased heart/body weight and right ventricular/heart	(1986)
m, f	104 weeks	Uf	6.6	54,912	32.0	—	—	weight ratios; lower left ventricular contractility; changes in	
		F[d]	—		32.0	—	—	blood chemistry; obstructive and restrictive lung disease; f: no	
		C	—		1.0	—	—	effects	
Rat, wistar, f;	19 h/day	Uf	4.24	48,336	12.5	1.5	3.1	Uf: decreased body wt in rats and mice but not hamsters;	Heinrich et al.
hamster, syrian, f;	5 days/week	F[d]	—	56,392	11.1	1.2	1.02	increased mortality, mice only; decreased lung compliance and	(1986a)
Mouse nmri, f	120 to	C	—		0.16	—	—	increased airway resistance, rats and hamsters; species	
	140 weeks							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	
Mouse, nmri, f,	18 h/day	Uf	4.5	40,365	14.2	2.3	2.8	Uf: increased lung wet weight starting at 3 mo	Heinrich et al.
c57bl/6n, f	5 days/week	F	0.01		14.2	2.9	2.4		(1995)
	23 mo	C	0.01		0.2	0.01	0.1	F: no noncancer effects reported	
	(nmri)								
	24 mo								
	(c57bl/6n)								

[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.

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

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

granulocytes (125 ± 39.7 versus 1.23 ± 1.14/μL in the controls). All values presented for this study are the mean with its standard deviation. The differences were significant for each cell type. There was also a small increase in lymphocytes (5.81 ± 4.72 versus 3.01 ± 1.23/μL in the controls).

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

Brightwell et al. (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Three exhaust dilutions were tested, producing concentrations of 0.7, 2.2, and 6.6 mg/m^3 DPM. The test animals (144 rats and 312 hamsters per exposure group) were exposed for five 16-h periods per week for 2 years. The four exposure types were gasoline, gasoline catalyst, diesel, and filtered diesel. The results presented were limited to statistically significant differences between exhaust-exposed and control animals. The inference from the discussion section of the paper was that there was a minimum of toxicity in the animals exposed to filtered diesel exhaust: "It is clear from the results presented that statistically significant differences between exhaust-exposed and control animals are almost exclusively limited to animals exposed to either gasoline or unfiltered diesel exhaust." Additional results are described in Section 5.1.2.3.

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

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

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

5.3. INTERACTIVE EFFECTS OF DIESEL EXHAUST

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

Mauderly et al. (1990a) compared the susceptibility of normal rats and rats with preexisting laboratory-induced pulmonary emphysema exposed for 7 h/day, 5 days/week for 24 mo to diesel exhaust containing 3.5 mg/m^3 DPM or to clean air (controls). Emphysema was induced in one-half of the rats by intratracheal instillation of elastase 6 weeks before exhaust exposure. Measurements included lung burdens of DPM, respiratory function, bronchoalveolar lavage, clearance of radiolabeled particles, pulmonary immune responses, lung collagen, excised lung weight and volume, histopathology, and mean linear intercept of terminal air spaces. None of the data for the 63 parameters measured suggest that rats with emphysematous lungs were more susceptible than rats with normal lungs to the effects of diesel exhaust exposure. In fact, each of the 14 emphysema-exhaust interactions detected by statistical analysis of variance indicated that emphysema acted to reduce the effects of diesel exhaust exposure. 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 DPM and radiolabeled particles ($t_{1/2}$ = 90 days versus 47 days for controls), and (5) increased lung weights. These effects were not seen in the developing rats. On a weight for weight (milligrams of DPM per gram of lung) basis, DPM accumulation in the lungs was similar in developing and adult rats immediately after the exposure. During the 6-month postexposure period, DPM clearance was much more rapid in the developing rats, approximately 2.5-fold. During postexposure, diesel particle-laden macrophages became aggregated in the developing rats, but these aggregations were located primarily in a subpleural position. The authors concluded that exposure to diesel exhaust, using pulmonary function, structural (qualitative or quantitative) biochemistry as the indices, did not affect the developing rat lung more severely than the adult rat lung.

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

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

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

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

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

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

There is some evidence indicating that species may differ in pulmonary responses to diesel exhaust (DE). Mauderly (1994) compared the pulmonary histopathology of rats and mice after 18 mo of exposure to DE. There was less aggregation of macrophages in rats. Diffuse septal thickening was noted in the mice, but there were few inflammatory cells, no focal fibrosis, little epithelial hyperplasia, and no epithelial metaplasia, as was observed in rats. Heinrich et al. (1986a) reported that wet lung weight of hamsters increased only 1.8-fold following chronic exposure to 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 Cynomolgus 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. 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 which dose/exposure rates of diesel exhaust (expressed in terms of the DPM concentration) result in injury to the lung and which appear to elicit no effect. The inhalation of poorly soluble particles, such as those found in diesel exhaust, increases the pulmonary particulate burden. When the dosing rate exceeds the ability of the pulmonary defense mechanisms to achieve a steady-state lung burden of particles, there is a slowing of clearance and the progressive retention of particles in the lung that can ultimately approach a complete cessation of lung clearance (Morrow, 1988). This phenomenon, which is reviewed in Section 3.4, particle overload, 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., 1981), 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 (Klosterkötter and Bünemann, 1961), volcanic ash (Wehner et al., 1986), amosite (Bolton et al., 1983), and manmade mineral fibers (Lee et al., 1988) among others. In more recent studies, animals have been exposed to 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.

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

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

The exact relationship between toxicity and particle size within the ultrafine particle mode, including DPM (BéruBé et al., 1999), remains unresolved. Studies reviewed in the PM CD (U.S. Environmental Protection Agency, 1996) suggest a greater inherent potential toxicity of inhaled ultrafine particles. Exposure to ultrafine particles may increase the release of proinflammatory mediators that could be involved in lung disease. For example, Driscoll and Maurer (1991) compared the effects of fine (0.3 um) and ultrafine (0.02 um) TiO2 particles instilled into the lungs of laboratory rats. Although both size modes caused an increase in the numbers of AMs and PMNs in the lungs, and release of TNF and fibronectin by AMs the responses were greater and more persistent with the ultrafine particles. While fine particle exposure resulted in a minimally increased prominence of particle-laden macrophages associated with alveolar ducts, ultrafine particle exposure produced a somewhat greater prominence of macrophages, some necrosis of macrophages and slight interstitial inflammation of the alveolar duct region. Moreover, collagen increased only with exposure to ultrafine particles.

Oberdörster et al. (1992) compared the effects of fine (0.25 um) and ultrafine (0.02 um) TiO2 particles instilled into the lungs of laboratory rats on various indicators of inflammation. Instillation of ultrafine particles increased the number of total cells recovered by lavage, decreased the percentage of AMs, and increased the percentage of PMNs and increased protein. Instillation with fine particles did not cause statistically significant effects. Thus, the ultrafine particles had greater pulmonary inflammatory potency than did larger sizes of this material. The investigators attributed the enhanced toxicity to greater interaction of the ultrafine particles with their large surface area, with alveolar and interstitial macrophages, which resulted in enhanced release of inflammatory mediators. They suggested that ultrafine particles of low in vitro solubility appear to enter the interstitium more readily than do larger sizes of the same material, which accounted for the increased contact with macrophages in this compartment of the lung. Driscoll and Maurer (1991) noted that the pulmonary retention of ultrafine TiO2 particles instilled into rat lungs was greater than for the same mass of fine mode TiO2 particles. Thus, the available evidence tends to suggest a potentially greater toxicity for inhaled ultrafine particles.

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

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

5.6. SUMMARY AND DISCUSSION

5.6.1. Effects of Diesel Exhaust on Humans

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

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

5.6.2.1. *Effects on Survival and Growth*

The data presented in Table 5-3 show limited effects on survival in mice and rats and some evidence of reduced body weight in rats following chronic exposures to concentrations of 1.5 mg/m^3 DPM or higher and exposure durations of 16 to 20 h/day, 5 days/week for 104 to 130 weeks. Increased lung weights and lung to body weight ratios in rats, mice, and hamsters;

an increased heart to body weight ratio in rats; and decreased lung and kidney weights in cats have been reported following chronic exposure to diesel exhaust. No evidence was found of an effect of diesel exhaust on other body organs (Table 5-4). The lowest-observed-effect level in rats approximated 1 to 2 mg/m^3 DPM for 7 h/day, 5 days/week for 104 weeks.

5.6.2.2. *Effects on Pulmonary Function*

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

5.6.2.3. *Histopathological and Histochemical Effects*

Histological studies have demonstrated that chronic exposure to diesel exhaust can result in effects on respiratory tract tissue (Table 5-6). Typical findings include alveolar histiocytosis, AM aggregation, tissue inflammation, increase in PMNs, hyperplasia of bronchiolar and alveolar Type II cells, thickened alveolar septa, edema, fibrosis, and emphysema. Lesions in the trachea and bronchi were observed in some studies. Associated with these histopathological findings were various histochemical changes in the lung, including increases in lung DNA, total protein, alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase; increased synthesis of collagen; and release of inflammatory mediators such as leukotriene LTB and prostaglandin $PGF_{2\alpha}$. Although the overall laboratory evidence is that prolonged exposure to DPM results in histopathological and

histochemical changes in the lungs of exposed animals, some studies have also demonstrated that there may be a threshold of exposure to DPM below which pathologic changes do not occur. These no-observed-adverse-effect levels for histopathological effects were reported to be 2 mg/m^3 for cynomolgus monkeys (the only concentration tested), 0.11 to 0.35 mg/m^3 for rats, and 0.25 mg/m^3 DPM for guinea pigs exposed for 7 to 20 h/day, 5 to 5.5 days/week for 104 to 130 weeks.

5.6.2.4. *Effects on Airway Clearance*

The pathological effects of DPM appear to be strongly dependent on the relative rates of pulmonary deposition and clearance (Table 5-7). Clearance of particles from the alveolar region of the lungs is a multiphasic process involving phagocytosis by AMs. Chronic exposure to DPM concentrations of about 1 mg/m^3 or above, under varying exposure durations, causes pulmonary clearance to be reduced with concomitant focal aggregations of particle-laden AMs, particularly in the peribronchiolar and alveolar regions, as well as in the hilar and mediastinal lymph nodes. The exposure concentration at which focal aggregates of particle-laden AMs occur may vary from species to species, depending on rate of uptake and pulmonary deposition, pulmonary clearance rates, the relative size of the AM population per unit of lung tissue, the rate of recruitment of AMs and leukocytes, and the relative efficiencies for removal of particles by the mucociliary and lymphatic transport system. The principal 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 (Health Effects Institute, 1995). Morrow (1988) suggested that the inability of particle-laden AMs to translocate to the mucociliary escalator is correlated to an average composite particle volume per AM in the lung. When this particle volume exceeds approximately 60 μm^3 per AM in the Fischer 344 rat, impairment of clearance appears to be initiated. When the particulate volume exceeds approximately 600 μm^3 per cell, evidence suggests that AM-mediated particulate clearance virtually ceases 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 5-14). Exposure caused a decreased level of spontaneous locomotor activity and a detrimental effect on learning in adulthood. In agreement with the behavioral changes was physiological evidence for delayed neuronal maturation. Exposures were to 6 mg/m^3 DPM for 8 h/day, 7 days/week from birth to about 7, 14, 21, or 28 days of age.

5.6.2.6. *Effects on Immunity and Allergenicity*

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

As with humans, there are animal data suggesting that DPM is a possible factor in the increasing incidence of allergic hypersensitivity. The effects have been demonstrated primarily in acute human and laboratory animal studies and appear to be associated mainly with the organic fraction of DPM. It also appears that synergies with DPM may increase the efficacy of known allergens. Both animal and human cell culture studies suggest that DPM also has the potential to act as an adjuvant.

5.6.2.7. *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 5-11 through 5-13 and 5-15).

5.6.3. Comparison of Filtered and Unfiltered Diesel Exhaust

The comparison of the toxic responses in laboratory animals exposed to whole diesel exhaust or filtered exhaust containing no particles demonstrates across laboratories that diesel particles are the principal etiologic agent of noncancerous health effects in laboratory animals exposed to diesel exhaust (Table 5-16). Whether the particles act additively or synergistically with the gases cannot be determined from the designs of the studies. Under equivalent exposure regimens, hamsters have

lower levels of retained DPM in their lungs than rats and mice do and consequently less pulmonary function impairment and pulmonary pathology. These differences may result from a lower intake rate of DPM, lower deposition rate and/or more rapid clearance rate, or lung tissue that is less susceptible to the cytotoxicity of DPM. Observations of a decreased respiration in hamsters when exposed by inhalation favor lower intake and deposition rates.

5.6.4. Interactive Effects of Diesel Exhaust

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

5.6.5. Conclusions

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

- The primary acute (high-concentration, short-term) effects of DE in humans include irritation, mild airway inflammation, and indicators of mild inflammation in lung lavage fluids. Allergenic effects also have been demonstrated under short-term exposure scenarios to either DE or DPM; the toxicological significance of these effects has yet to be resolved.
- Noncancer effects in humans from long-term chronic exposure to DPM are not evident. Noncancer effects from long-term exposure to DPM of several laboratory animal species include pulmonary histopathology and inflammation.

Although the mode of action of DE/DPM is not clearly evident for any of the effects documented in this chapter, the respiratory tract effects observed under acute scenarios are suggestive of an irritant mechanism, while lung effects observed in chronic scenarios indicate an underlying inflammatory response. Current knowledge indicates that the carbonaceous core of the diesel particle is the causative agent of the lung effects, with the extent of the injury being mediated

at least in part by a progressive impairment of alveolar macrophages. It is noted that lung effects occur in response to DPM exposure in several species and occur in rats at doses lower than those inducing particle overload and a tumorigenic response (see above); it follows that lung effects such as inflammation and fibrosis are relevant in the development of risk assessments for DPM.

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6.1. INTRODUCTION—BACKGROUND OF THE INHALATION RfC AND ORAL RfD

Construction of a risk assessment for a toxicant requires several steps, including synthesis of information into a coherent reasonable evaluation of the hazard it presents to humans and definition of the relationship between dose of the substance and the resultant biological response. The EPA's vehicle for construction of these vital portions of a risk assessment, hazard identification and dose-response, is the inhalation reference dose (RfD) for an orally ingested toxicant or the inhalation reference concentration (RfC) for an inhaled airborne toxicant.

This chapter explains the concept and structure of the RfC as the Agency's estimate of a "safe" level, and utilizes the information documented in Chapter 5 to synthesize this estimate for diesel.

6.1.1. The Acceptable Daily Intake

Since its inception, EPA has advocated critical evaluation of data related to noncancer toxicity of compounds. When possible, quantitative estimates were calculated from combining effect levels, such as a no-observed-adverse-effect-level (NOAEL) or a lowest-observed-adverse-effect-level (LOAEL), with certain "safety factors" into an Acceptable Daily Intake (ADI). Such procedures have a wide and historical basis; the National Research Council (NRC) recommended the ADI approach in 1977 to characterize levels of pollutants in drinking water with respect to human health (NRC, 1977, 1980). These approaches, as well as the oral reference dose (RfD) and inhalation reference concentration (RfC) discussed below, are based on the assumption that a threshold exists for the human population below which no effect will occur. Basically, all of these approaches attempt to identify an estimate of a likely subthreshold concentration.

6.1.2. Oral RfD and Inhalation RfC—Dose-Response Assessments Inclusive of Uncertainty Factors

The National Academy of Sciences (NAS) report entitled "Risk Assessment in the Federal Government: Managing the Process" was issued in 1983 (NRC, 1983). Among the many fundamental concepts and principles put forth in this report was the recommendation that scientific aspects be explicitly separated from policy issues in the risk assessment process.

EPA's response included development of the RfD and guidelines on its derivation (Barnes and Dourson, 1988) and subsequent development of the parallel inhalation RfC and its formal methodology (U.S. EPA, 1994). The definition of the inhalation RfC is:

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 an appreciable risk of deleterious noncancer effects during a lifetime.

Similar to ADIs in intent, RfC/Ds are dose-response assessments for noncancer effects based upon a more rigorous methodology adhering to the principles set forth in the 1983 NRC report. The RfC methodology includes guidance on the consistent application to effect levels of "uncertainty factors" (UFs) rather than the ADI "safety factors " for extrapolations.

The basic quantitative formula for derivation of an RfC, given in Equation 6-1, has as its basic components an effect level and UFs. The units of an RfC are mg/m^3.

$$RfC = \frac{NOAEL}{UF} \qquad (6\text{-}1)$$

The concept of an effect level, such as the NOAEL or LOAEL, is consistent with the ADI construct. Alternatively, the benchmark dose/concentration (BMC) approach may be used as the effect level in Equation 6-1. The BMC approach applies a line-fitting model to the key data and then uses the dose-response relationship to interpolate an exposure concentration that is predicted to result in a predefined level of response (BMR), such as a 10% incidence of a lesion. The lower confidence limit on the concentration predicted to result in the BMR is designated the BMC and would be the numerator in Equation 6-1.

6.1.3. UFs—Designation and Application

The UFs, their components, and their intended usage in the RfC methodology are given in Table 6-1. As can be seen, they are fitted to the RfC definition providing consideration for *lifetime* exposure (subchronic-to-chronic duration factor) for *sensitive subgroups* (human-to-sensitive human factor) within the *human population* (animal-to-human extrapolation factor). Consideration for effect levels (a LOAEL to a NOAEL extrapolation factor) and a database factor are also part of the RfC methodology. The default values for the A and H UF are also shown with their pharmacokinetic (PK) and pharmacodynamic (PD) components, each at $10^{0.5}$, which is rounded to 3 when applied singly. The pharmacokinetic adjustments to dose provided for in derivation of RfCs (EPA, 1994) allow for application of only the PD component of this UF.

As with the safety factors for the ADI, UFs for the RfD/C are applied in a multiplicative manner. Unlike safety factors, which are almost always applied to effect levels as even factors of

10, UFs may be applied to effect levels as partial values of 10, e.g., $10^{0.5}$ (rounded to 3) or 1, based on the circumstances. An example of application of a partial UF is for animal-to-human extrapolation with dosimetry adjustments as explained below in Section 6.1.4.

6.1.4. Animal-to-Human Extrapolation Factor in the RfC—A Human Equivalent Concentration

A major difference exists between the oral RfD and inhalation RfC in the animal-to-human (A) extrapolation procedure. Table 6-1 indicates that the A UF may have the default value of 10 and, furthermore, that this factor may be differentiated into pharmacokinetic (PK; dose to tissue) and pharmacodynamic (PD; tissue response) components. Adjustments to the externally applied factors may be made to address the PK component of this UF. The RfC methodology (U.S. EPA, 1994) provides models and procedures for adjustments with both particles and gases. In this assessment, several pharmacokinetic models, some capable of adjusting for all aspects of the PK component such as absorption, uptake, and clearance, are reviewed and evaluated. The goal of these adjustments is to derive an external concentration that would produce the same internal tissue dose in humans as in animals, i.e., to produce a Human Equivalent Concentration (HEC) from the animal effect level. When this adjustment is made, the quantitative pharmacokinetics are considered the same and the PK component of this UF is addressed. This adjustment for dosimetry is accommodated by application of a partial UF for interspecies extrapolation of $10^{0.5}$ for the remaining uncertainty about the PD component. When applied singly this factor, by policy, is rounded to 3.

Table 6-1. UFs and their default values used in EPA's noncancer RfD and RfC methodology

UF—Area of extrapolation	Default values
A—animal-to-human	10 ($10^{0.5}$ PK × $10^{0.5}$ PD)
H—human-to-sensitive human	10 ($10^{0.5}$ PK × $10^{0.5}$ PD)
S—subchronic-to-chronic	10
L—LOAEL-to-NOAEL	10
D—incomplete-to-complete data	10

6.1.5. Basic Procedures for Derivation of an RfC—Identification of the Critical Effect, the Principal Study, Application of UF, and Assignment of Confidence Level

The goal of the RfC/D methodologies is to provide rationale and guidance on a quantitative approach in evaluating toxicity data to derive a dose-response assessment. Equation 6-1 is a condensation of the RfC process and serves as a basis for discussing the procedures for its derivation. Having a NOAEL for this equation implies that a specific adverse effect has been identified and that there is documentation that this effect does not occur at this particular concentration, i.e., the NOAEL.

RfC derivation provides for evaluation of the toxicity database to identify a "critical effect," which is defined as "the first adverse effect, or its known precursor, that occurs as the dose rate increases." Analysis of the database also allows for choice of a "principal study," "the study that contributes most significantly to the qualitative and quantitative risk," in characterizing the dose-response of the critical effect. To fulfill the definition of the RfC, the critical effect would have to be consonant with the definition of the RfC given above, e.g., relevant to humans and observed under chronic, long-term conditions. Other studies that are pertinent to identifying the dose-response or threshold for the effect are included in the derivation as supporting studies. Thus, the NOAEL in Equation 6-1 would be based on the absence of the critical effect as documented in the principal study.

Assignment of an appropriate UF would be accomplished in consideration of the information available on the specific chemical as per Table 6-1. General guidelines were discussed briefly in this introductory section and are discussed at length in the RfC Methods (U.S. EPA, 1994). As explained above, assignment of specific values of UF may have both policy and science implications. General policy is to provide clear explanatory text with each UF assignment. Composite UF values vary widely. In cases where information on the NOAEL is well defined in a known sensitive subgroup of humans, the UF may be 1. With sparse information, UF values have ranged up to 3000. If none of the areas of extrapolation in Table 6-1 are addressed (i.e., all areas of uncertainty are applicable), then no RfC is derived.

Confidence statements are synthesized for each RfC. They are meant to serve as a repository for statements that clearly communicate associated uncertainties, establish and dichotomize policy from scientific bases, make clear specific limitations and strengths, and express any other concerns reflecting on the overall quality of the assessment (U.S. EPA, 1994; Ohanian et al., 1997). The RfC/D methodologies allow for high, medium, and low levels of confidence, with the level being assigned subsequent to an analysis as above. Levels are surmised for both the overall database and the principal study/ies, with the database confidence taking precedence over that assigned to the study. In general, the level of confidence is inversely

related to both the composite UF and the likelihood that the RfC would change with the availability of new information; an RfC based on a sensitive effect in a sensitive human subgroup as reported in a exemplary study with a composite UF of < 30 would more than likely be one of high confidence.

6.2. ISSUES IN DERIVATION OF THE DIESEL RfC

Information available on diesel particulate matter (DPM) is that in other databases and therefore includes several areas of controversy and uncertainty. This section introduces issues concerning DPM. Subsequent sections will then more fully examine and consider these issues.

6.2.1. Chronic Noncancer Effects in Humans—Relevancy of Rodent Data

Current information shows that humans and rodents share some noncancer responses to poorly soluble particles such as DPM that are qualitatively similar. These analogous responses suggest that a potential commonality exists between humans and rodents in the underlying mode(s) of action of DPM. These analogous responses and shared steps in the mode of action do not appear to extend to the tumorigenic response seen in one particular rodent species, the rat. As discussed in other sections of this document, the relevance to humans of the tumorigenic response in rat lungs occurring under particle overload conditions is problematic.

6.2.2. Pulmonary Pathology and Immunologic Effects as Critical Effects

Recent investigations in both laboratory animals and humans in clinical settings have associated exposure to DPM with immunologic effects, especially enhanced allergenicity. The relationship between pulmonary histopathology and allergenic effects is compared and contrasted in the choice of pulmonary histopathology as a scientifically defensible critical effect upon which to base this assessment.

6.2.3. Application of UFs

As discussed above, applications of UF consider both science and policy. Because of the extensive database of well-conducted long-term chronic studies in several species, much is known about the effects of DPM on the lung as target organ. Relatively few areas of uncertainty are applicable to the diesel database. Moreover, the application of a pharmacokinetic model in this assessment obviates a portion of the animal-to-human UF as explained above. Questions concerning the application of uncertainty for consideration of the enhanced allergenic effects are presented and discussed.

6.2.4. Relationship of DPM to Ambient Levels of $PM_{2.5}$

DPM is acknowledged as a component of the fine particulate matter ($PM_{2.5}$) present in ambient air, especially in urban areas. It is known that compared with $PM_{2.5}$, DPM has a higher proportion of fine and ultrafine particles and a higher content of organic compounds absorbed onto the carbon core. DPM could thus be considered a subcategory of $PM_{2.5}$ with greater toxicologic potential from the higher organic compound content, which would penetrate more efficiently into the alveolar compartment because of the preponderance of small particles in DPM.

6.3. APPROACH FOR DERIVATION OF THE RfC FOR DIESEL ENGINE EMISSIONS

6.3.1. Consideration of Long-Term Inhalation Studies

Twelve long-term (>1 year) laboratory animal inhalation studies of diesel engine emissions have been conducted. These studies focused on effects in the pulmonary region. Studies 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 6.2) as well as pulmonary histopathology. Effects in the upper respiratory tract and other organs were not found consistently in chronic animal exposures.

6.3.2. Derivation of a HEC—Application of a Pharmacokinetic Model

PK models may be used to project across species concentrations of a toxicant that would result in equivalent internal doses. When used for these purposes, PK models may be termed dosimetric models. Chapter 3 reviewed and evaluated a number of dosimetric models applicable to DPM. The 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 use in this assessment. A major assumption in this model is 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 rat studies to human exposures. See

Chapter 3 for further discussion of the model and Appendix B for complete specifics on the use of the model.

A principal and critical decision in utilizing any dosimetric model is the measure of dose. DPM 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), the toxicity of DPM may result 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. 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 measure of dose. See Chapters 6 and 9 for further details.

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 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. It has also been shown, however, that the particle characteristics in a diesel exhaust exposure study depend very much on the procedures used to generate the chamber atmosphere. Because of the rapid coagulation of particles, the volume and temperature of the dilution gas are especially important. 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 representative DPM particle size of MMAD = 0.2 μm and $\sigma g = 2.3$ (values typically reported for DPM) for modeling of lung burden. For consistency, the lung burdens for the other studies were also calculated using this assumption. The difference in the HEC 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. CHOICE OF THE CRITICAL EFFECT—RATIONALE AND JUSTIFICATION

6.4.1. Mode-of-Action and Candidate Effects

Mode-of-action information about respiratory effects from diesel exposure indicates that the pathogenic sequence following the inhalation of diesel exhaust 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 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 like those described in the studies reviewed in Chapter 7. Epidemiologic studies of occupationally exposed people provide suggestive evidence for a respiratory effect. Although detailed information describing the pathogenesis of respiratory effects in humans is lacking, the effects reported in studies of humans exposed to diesel exhaust lend qualitative support to the findings in controlled animal studies and therefore to this basic mode of action.

Evidence from the available toxicological data on diesel exhaust consistently indicates 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, most of which are described and evaluated in Chapter 7. The endpoints of concern include biochemical, histopathological, and functional changes in the pulmonary and tracheobronchial regions.

The occurrence of a lung cancer response in rats under conditions of "clearance overload" from diesel exhaust/DPM has been discussed elsewhere in this document as being possibly unique to the rat and of problematic relevance to human lung responses. Yet effects in the rat lung are being proposed as the basis for the RfC. There are several reasons why these effects are considered valid and relevant for RfC derivation. First, the effects considered, inflammation (inflammatory cell infiltration) and fibrosis, are noncancer effects. Second, similar noncancer effects are seen in other species (mouse, hamster), albeit under conditions of higher exposure than rats, and these species do not manifest a cancer response. Third, rats and humans do exhibit similar noncancer responses (macrophage response and interstitial fibrosis) to less toxic particles (i.e., coal dust) and to lower concentrations of poorly soluble particles such as DPM. Thus, when viewed across species the pulmonary effects of inflammation and fibrosis are considered dissociable from the cancer response and of likely relevance to humans.

Some evidence suggests liver and kidney changes in animals exposed to diesel exhaust. There have also been some indications of neurotoxicity at high concentrations of diesel exhaust. These data, however, 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 potential hazard.

Recent evidence has accumulated for effects of diesel exhaust and DPM on respiratory system-related immune function, especially enhanced or exacerbated allergenicity. Chapter 5 describes studies of human cells in vitro as well as human nasal instillation and inhalation studies that have demonstrated the potential for DPM to enhance allergic inflammatory responses. This effect included observations wherein increases of IgE were produced in nasal lavage, especially when DPM was instilled concomitantly with allergen in atopic rhinitic subjects. DPM has also been shown to enhance histamine-induced increase of certain inflammatory mediators such as IL-8 and GM-CSF. Exposure of healthy human subjects to dilute diesel exhaust (300 μg) for 1 hour with intermittent exercise led to an acute mediator and cellular inflammatory response in the airways and peripheral blood.

6.4.2. Rationale and Justification

The choice of critical effect for DPM must be consonant with the definition given above and made in consideration of the purposes of the RfC, e.g., a lifetime continuous exposure that is without adverse effects. From the discussion above, the principal candidate critical effects are the pulmonary histopathological changes in rats and enhanced allergenic effects in the upper airways of animals and humans. The following points compare and contrast these effects:

- Pulmonary histopathology is shown consistently in several species with clear dose-response under long-term realistic exposure scenarios. Allergenic effects are shown consistently in both animal and clinical human studies but, dose-response and concentration × times (C × t) relationships are not available under any exposure scenario.

- The relevance of these candidate effects to humans is each subject to qualifications. Enhanced allergenic effects have been demonstrated in humans. However, the observations were mostly in sensitized individuals exposed via nasal instillation, a questionable route, and to relatively high bolus doses. The pulmonary histopathology observed in rat studies is only marginally supported by effects that may occur in humans.

- Events that stimulate inflammatory processes may underlie both these effects. Fibrogenesis is necessarily preceded and accompanied by inflammation. Events such as enhancement of inflammatory cytokines have been associated with allergenic enhancement.

As the RfC is a dose-response assessment for effects encountered under conditions of chronic exposure, pulmonary histopathology would therefore be the most robust and defensible choice for the critical effect. Long-term, dose-response, and mode-of-action information could warrant reconsideration of allergenic effects as being critical or possibly co-critical.

6.5. PRINCIPAL STUDIES FOR INHALATION RfC DERIVATION

The experimental protocol and results for the principal studies demonstrating and characterizing the critical effect are discussed in Chapter 7 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 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 histopathological 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 histopathological changes were seen, including focal areas of epithelial metaplasia. Fibrosis and metaplasia increased with 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. Histopathological 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, although measurements for all groups, including those of ITRI, are quite similar to one another. 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 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, histopathological, and functional changes in the pulmonary region at 0.35 mg/m^3 (LOAEL = 3.5 mg/m^3). The 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 6-2, 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.

6.6. SUPPORTING STUDIES FOR INHALATION RfC 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

Table 6-2. 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 obtained from applying the dosimetric model of Yu and Yoon (1990).

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 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 a 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 a LOAEL at 0.735 mg/m^3 and a 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. No fibrosis, focal emphysema, or inflammation was observed. The monkeys exposed to diesel exhaust demonstrated small-airway obstructive disease. This study demonstrates a 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 histopathological examination revealed different levels of response among the three species, histopathological effects were seen in all species and effects on pulmonary function were observed in rats and hamsters. This study demonstrates a 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 a 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 a 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 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 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 numbers 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, a 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 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 (discussed further below).

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 7. 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.

6.6.1. Respiratory Tract Effects in Species Other Than the Rat

In several of the chronic inhalation studies described in Chapter 7, one or more species other than the rat were also exposed and examined for toxic effects. These 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 histopathological 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 a LOAEL at 0.735 mg/m^3 and a 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 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 1.5-fold in hamsters, twofold in mice, and threefold 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 of noncancer effects in rats compared with other rodent species. 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 histopathologically 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 a qualitatively different response in rats. This comparison is weakened because 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 a 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^2, respectively. Because the dose spacing is so wide in the ITRI study, the Kaplan study is more appropriate for defining a 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 (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.

6.6.2. 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 BMC 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 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 chronically exposed 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.

Several key issues concerning the derivation and interpretation of BMCs, especially in a comparative manner over a variety of studies with a myriad of endpoints with differing types of data such as with diesel, are yet to be resolved by the Agency. Several principal limitations are the following:

- Some key studies in rats have inadequate quantitative data for BMC.
- The scientific criteria for selecting BMC from many endpoints remains to be established.
- A deposition model is available only for rats (it is not clear how to compare BMCs based on deposition/retention models with BMCs 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. DERIVATION OF THE INHALATION RfC

6.7.1. The Effect Level—A NOAEL From a Chronic Inhalation Study

Based on the analysis above, the studies of chronic exposures to diesel emissions performed at ITRI and HERP (Ishinishi et al., 1988; Mauderly et al., 1988) were selected as the basis of the RfC, because they identify both a NOAEL and a LOAEL for rats exposed chronically, because they identified the highest NOAEL (Table 6-2), and because they are thoroughly reported. The only other study identifying both a NOAEL and a 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, are also contributory to 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, these data are not sufficiently robust for derivation of an RfC but may be used as supporting information. The pulmonary effects, including histopathological 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 (nasopharyngeal) region of the respiratory system or in other organs at the lowest levels that produces pulmonary effects in chronic exposures. The exposure concentration of 0.46 mg/m^3 from the study of Ishinishi et al. (1988) is the NOAEL. Application of the dosimetric model of Yu and Yoon (1990) to this value resulted in a NOAEL(HEC) of 0.155 mg/m^3.

6.7.2. Application of UFs—Animal-to-Human and Sensitive Subgroups

Principal areas of uncertainty for this assessment are the human-to-sensitive human and animal-to-human extrapolations (Table 6-1). Because the RfC is based on a NOAEL from a chronic animal study, neither LOAEL-to-NOAEL nor subchronic-to-chronic extrapolations are needed. Also, the database for diesel is robust, with numerous well-conducted chronic studies in addition to information showing no adverse effects on development in two species or on reproduction in a two-generational study, all of which serve to eliminate the need for a UF for database deficiencies.

No quantitative information exists regarding subgroups that may be sensitive to the effects of diesel exhaust or DPM. The information available on enhanced allergenic effects discussed above and in Chapter 7 suggests that individuals already sensitized by various antigens are more sensitive to exposure to DPM than are those who are not, especially when undergoing an allergenic inflammatory episode. However, no quantitation of the relative sensitivity is available. Nor is there information indicating that children or male or female neonates are especially more or less sensitive. Therefore the default value of 10 is used to accommodate human-to-sensitive human extrapolation (Table 6-1).

Several issues reside in applying the UF for animal-to-human extrapolation to the diesel database. First, the PK component of this UF (see Table 6-1) has been addressed by the application of a dosimetric model to obtain a HEC, thereby decreasing the UF to 3 (or $10^{0.5}$) for the residual PD component. Second, information discussed above and in Chapter 6 indicates that for certain endpoints such as chronic inflammation, the rat appears to have a more sensitive response than other species, including humans. That rats are more sensitive to the effects of inhaled DPM than are humans could be considered evidence sufficient to eliminate the remaining PD component of this UF. However, mode-of-action evidence for the various effects observed with diesel, especially pulmonary histopathology and immunologic effects such as enhanced allergenicity, indicate that events stimulatory to inflammatory processes underlie these effects, i.e., neutrophilic inflammation preceding fibrogenesis and such events as increased cytokine production preceding immunologic effects. Although indications are that humans are less sensitive than are rats to the inflammatory-mediated endpoint of fibrogenesis, it is problematic to

presume that humans would also be less sensitive to other inflammatory-mediated endpoints such as enhanced allergenicity that are now documented in the literature. In consideration of this missing specific mode-of-action information on inflammation, the PD component is retained at the value of 3.

The total composite UF is therefore $10 \times 3 = 30$.

$$\text{The resultant RfC} = \frac{\text{NOAEL(HEC)}}{\text{UF}} = \frac{0.155\ \text{mg/m}^3}{30} = 5\text{E-}3\ \text{mg/m}^3\ (5\ \mu\text{g/m}^3)$$

6.7.3. Designation of Confidence Level

The studies used as the basis for the RfC were well-conducted chronic studies with adequate numbers of animals, in which the target tissues (i.e., the respiratory tract) were thoroughly examined and in which the LOAELs and NOAELs were consistent across studies. The database contains several chronic studies, including multiple species, that support the LOAEL observed in the principal study. The availability of multiple chronic studies all having consistent effect levels imparts a high confidence to the principal study. Developmental and multigeneration reproductive studies also exist, resulting in a high-confidence database. The endpoints chosen have relevancy to the human response to other poorly soluble particulates.

The modeling employed in this assessment to derive HECs includes both deposition and clearance mechanisms, although assumptions have been made with certain of the clearance parameters. Current mode-of-action information indicates that events stimulatory to inflammatory processes underlie the effects reported in the pulmonary (target) tissues. Continued investigation in this area may clarify the status of other effects (e.g., immunologic) reported from diesel exposure.

The application of this RfC to general ambient particulate matter such as $PM_{2.5}$ must be limited. Compared with $PM_{2.5}$, DPM has a relatively high organic content and a preponderance of small particles capable of penetrating to the lung. As a consequence, DPM may be considered a subcategory of $PM_{2.5}$, with perhaps a greater potential for eliciting toxicity.

High confidence in both the studies and database leads to high confidence in the RfC itself.

6.8. SUMMARY

Table 6-3 summarizes the principal decision points in derivation of the diesel RfC, the Agency's estimate of a continuous inhalation exposure that is considered to be without an appreciable risk of deleterious noncancer effects during a lifetime.

Table 6-3. Decision summary for the derivation of the RfC for diesel engine emissions

Critical effect	Pulmonary histopathology in rats
Principal study	Ishinishi et al., 1988; Mauderly et al., 1988
NOAEL	0.46 mg/m^3
Model adjusted NOAEL = NOAEL(HEC)	0.155 mg/m^3
UFs	10—Human-to-sensitive human 3—Animal-to-human (pharmacodynamics)
Composite UF	30
NOAEL(HEC) / UF = RfC	0.155 mg/m^3 / 30 = 5E-3 mg/m^3
Confidence in the RfC	High

The derivation of this RfC was made in consideration of several candidate critical effects (including immunologic endpoints), in consideration of the relevancy of the critical effect chosen to the human response, and in recognition of the strengths and limitations of the modeling applied to obtain a human equivalent concentration (HEC).

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7. CARCINOGENICITY OF DIESEL EXHAUST

7.1. INTRODUCTION

Initial health hazard concerns regarding the potential carcinogenicity of diesel exhaust were based on the reported induction of skin papillomas by diesel particle extracts (Kotin et al., 1955), evidence for mutagenicity of extracts (Huisingh et al., 1978), evidence that components of diesel extract act as weak tumor promoters (Zamora et al., 1983), and the knowledge that diesel particles and their associated organics are respirable. During the 1980s, both human epidemiology studies and long-term animal cancer bioassays were initiated. In 1981, Waller published the first epidemiologic investigation, a retrospective mortality study of London transport workers. Since then a large number of cohort and case-control studies have been carried out with railroad workers, dockworkers, truck drivers, construction workers, and bus garage employees. During 1986 and 1987, several chronic animal cancer bioassays were published. These and numerous laboratory investigations carried out since then have been directed toward assessing the carcinogenic potential of whole exhaust, evaluating the importance of various components of exhaust in the induction of cancer, and understanding the mode of action and implications of deposition, retention, and clearance of diesel exhaust particles.

The purpose of this chapter is to evaluate the carcinogenic potential of diesel exhaust in both animals (Section 7.3) and humans (Sections 7.1 and 7.2), determine likely mode/s of action (Section 7.4), and provide an overall weight-of-evidence (Section 7.5) for carcinogenicity in humans. This assessment focuses on diesel exhaust, although diesel particles comprise a portion of all ambient particulate matter (PM). Although PM, notably PM_{10} (PM $\leq \mu m$ in diameter), has been identified for many years as potentially impacting human health, these effects have been evaluated in a separate document (EPA, 1996). This document is also undergoing revision.

In this section, 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 followup 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 9 cohort mortality studies (one of the mortality studies also included a nested lung cancer case-control study), 10 lung cancer case-control studies, and 7 bladder cancer case-control studies are considered in this section.

7.2. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EXHAUST

7.2.1. Cohort Studies

7.2.1.1. *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 midpoint of the 25-year followup 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.

7.2.1.2. *Howe et al. (1983): Cancer Mortality (1965 to 1977) in Relation to Diesel Fumes 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 deaths 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 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.

7.2.1.3. *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, however. 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 with 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 followup 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 followup (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.

7.2.1.4. *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 followup period was 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 followup (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 followup 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 followup 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.

7.2.1.5. *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 followup) was divided into three exposure categories: (1) clerks with 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.

7.2.1.6. *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 followup. 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 followup, 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.

7.2.1.7. *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 had 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 had 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.

7.2.1.8. *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 followup, 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, SMR 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.

7.2.1.9. *Hansen (1993): A Followup 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. ICD Revision 8 was used to code the underlying cause of death.

Test-based CIs 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 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 followup 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 7-1 summarizes the foregoing cohort studies.

7.2.2. Case-Control Studies of Lung Cancer

7.2.2.1. *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

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Waller (1981)	Approximately 20,000 male London transportation workers Aged 45 to 64 years 25 years followup (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

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
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 followup (i.e., not enough time for lung cancer latency) No adjustment for confounders such
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 followup 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.

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Edling et al. (1987)	694 male bus garage employees Followup 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 (1983)	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 followup	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 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	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 followup (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 (OF 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

Table 7-1. Epidemiologic studies of the health effects of exposure to diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
Hansen (1993)	Cohort of 57,249 unskilled laborers, ages 15 to 74, in Denmark (nationwide census file) November 9, 1970 Followup 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; OF = occupationally active; GP = general population.

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 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 (odds ratio [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. Furthermore, 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.

7.2.2.2. *Hall and Wynder (1984): A Case-Control Study of Diesel Exhaust Exposure and Lung Cancer*

Hall and Wynder (1984) 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 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. Furthermore, 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.

7.2.2.3. *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.

7.2.2.4. *Lerchen et al. (1987): Lung Cancer and Occupation in New Mexico*

This is a population-based case-control study conducted in New Mexico that examined 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. No excess risks were detected 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.

7.2.2.5. *Garshick et al. (1987): A Case-Control Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers*

An earlier pilot study of the mortality of railroad workers by the same investigators (Schenker et al., 1984) found a moderately high risk of lung cancer among the workers who were

exposed to diesel exhaust compared with 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.

7.2.2.6. *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 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.

7.2.2.7. ***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 from 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.

7.2.2.8. *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 dockworkers. 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.

7.2.2.9. *Steenland et al. (1998): Diesel Exhaust and Lung Cancer in the Trucking Industry: Exposure-Response Analyses and Risk Assessment*

Steenland et al. (1998) conducted an exposure-response analysis by supplementing the data from their earlier case-control study of lung cancer and truck drivers in the Teamsters Union (Steenland et al., 1990) with exposure estimates based on a 1990 industrial hygiene survey of elemental carbon exposures a surrogate for diesel exhaust in the trucking industry.

Study subjects were long-term Teamsters enrolled in the pension system who died during the period 1982-1983. Using death certificate information, the researchers identified 994 cases of lung cancer for the study period, and 1,085 non lung cancer deaths served as controls. Subjects were divided into job categories based on the job each held the longest. Most had held only one type of job. The job categories were short-haul driver, long-haul driver, mechanic, dockworker, other jobs with potential diesel exposure, and jobs outside the trucking industry without occupational diesel exposure. Smoking histories were obtained from next of kin. Odds ratios were calculated for work in an exposed job category at any time and after 1959 (an estimated date when the majority of heavy duty trucks had converted to diesel) compared with work in nonexposed jobs. Odds ratios were adjusted for age, smoking, and potential asbestos exposure. Trends in effect estimates for duration of work in an exposed job were also calculated.

An industrial hygiene survey by Zaebst et al. (1991) of elemental carbon exposures in the trucking industry provided exposure estimates for each job category in 1990. The elemental carbon measurements were generally consistent with the epidemiologic results, in that mechanics are found to have the highest exposures and relative risk, followed by long-haul and then short-haul drivers, although dockworkers have the highest exposures and the lowest relative risks.

Past exposures were estimated assuming that they were a function of (1) the number of heavy-duty trucks on the road, (2) the particulate emissions (grams/mile) of diesel engines over time, and (3) leaks from truck exhaust systems for long-haul drivers. Estimates of past exposure to elemental carbon, as a marker for diesel exhaust exposure, for subjects in the case-control study were made by assuming that average 1990 levels for a job category could be assigned to all subjects in that category, and that levels prior to 1990 were directly proportional to vehicle miles traveled by heavy-duty trucks and the estimated emission levels of diesel engines. A 1975 exposure level of elemental carbon in term of micrograms per cubic meter was estimated by the following equation: 1975 level = 1990 level*(vehicle miles 1975/vehicle miles 1990) (emissions 1975/emissions 1990). Once estimates of exposure for each year of work history were derived for each subject, analyses were conducted by cumulative level of estimated carbon exposure.

Estimates were made for long-haul drivers (n = 1,237), short-haul drivers (n = 297), dockworkers (n = 164), mechanics (n = 88), and those outside the trucking industry (n = 150). Logistic regression was used to estimate odds ratios adjusted for five categories of age, race, smoking (never, former-quitting before 1963, former-quitting in 1963 or later, current-with <1 pack per day, and current-with 1 or more packs per day), diet, and reported asbestos exposure. A variety of models for cumulative exposure were considered, including a log-linear model with cumulative exposure, a model adding a quadratic term for cumulative exposure, a log transform of cumulative exposure, dummy variables for quartile of cumulative exposure, and smoothing splines of cumulative exposure. The estimates of rate ratios from logistic regression for specific levels of exposure to elemental carbon were then used to derive excess risk estimates for lung cancer after lifetime exposure to elemental carbon.

The log of cumulative exposure was found to be the best fitting model and was a significant predictor ($p = 0.01$). Odds ratios for quartile of cumulative exposure show a pattern of significantly increasing trends in risk with increasing exposure, ranging between 1.08 and 1.72, depending on the exposure level and lag structure used. The lifetime excess risk of lung cancer death (through age 75) for a male truck driver was estimated to be in the range of 1.4-2.3% (95% confidence limits ranged from 0.3% to 4.6%) above the background risk, depending on the emissions scenarios assumed. The authors conclude that the data suggest a positive and significant increase in lung cancer risk with increasing estimated cumulative exposure to diesel exhaust among workers in the trucking industry. They assert that these estimates suggest that the lifetime excess risk for lung cancer is 10 times higher than the OSHA standards, but caution that the results should be viewed as exploratory.

The authors acknowledge that the increasing trend in risk with increasing estimates of cumulative exposure is partly due to the fact that a component of cumulative dose is simple

duration of exposure, and that analyses by simple duration also exhibit a positive trend with duration. This analysis essentially weights the duration by contrived estimates of exposure intensity, and they acknowledge that this weighting depends on very broad assumptions.

This is not an analysis of new data that provides independent estimates of relative risk for diesel exhaust and lung cancer incidence. Instead, it is an attempt to convert the data from Steenland's earlier study of lung cancer for the purpose of estimating a different risk metric, "lifetime excess risk of lung cancer," by augmenting these data with limited industrial hygiene data and rationalizations about plausible models for cumulative exposure.

The Health Effects Institute (HEI, 1999) and others have raised some questions about the exposure estimations and control for confounding variables. EPA and NIOSH will address these concerns in the year 2000. It should be noted that these concerns are about the use of these data for quantitative risk assessment. As far as qualitative risk assessment is concerned, this study is still considered to be positive and strong.

7.2.2.10. *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 1 control, whereas 2,515 were matched to 2 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.

7.2.2.11. *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 dockworkers 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 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 the 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 up to 2 years before 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 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, methodological limitations to this study 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 dockworkers 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 7-2 summarizes the above lung cancer case-control studies.

7.2.3. Case-Control Study of Prostate Cancer

7.2.3.1. *Aronsen et al. (1996): Occupational Risk Factors for Prostate Cancer: Results from a Case-Control Study in Montreal, Quebec, Canada*

A population-based case-control study was undertaken in 1979 to explore possible associations between many types of cancer and hundreds of occupational exposures. The current report provides a more refined analysis focusing only on prostate cancer and those exposures that showed associations with this site in the original analysis.

A total of 557 cases of incident, histologically confirmed prostate cancer were identified among males aged 35 to 70 years resident in the Montreal area. The timeframe for eligibility of incident cancers was not provided. Of these, 449 (81%) subjects were interviewed. Two sets of controls were used. Out of 740 population controls, 533 (72%) persons identified by random digit dialing or electoral lists provided interview data. Additionally, 1,550 controls were selected from the non-prostate cancer cases identified in the original study. Both control groups were pooled for the analysis after determining that the estimates did not depend on the control group.

The exposure data were obtained by interview questionnaire, with a structured section requesting information on potential confounders and a semistructured probing section designed to obtain a detailed description of each job the subject had in his working lifetime. A team of chemists and hygienists translated each job into a list of potential exposures by means of a checklist of 294 substances. The current analysis focused on 17 occupations, 11 industries, and

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	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 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	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
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

Table 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
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 7-2. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of lung cancer

Authors	Population studied	Diesel exhaust exposure	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 dockworkers	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.

27 substances as exposures. Unconditional logistic regression models were used to provide effect estimates adjusted for potential confounding by nonoccupational variables such as age, family income, ethnicity, Quetelet index, and respondent status. Diesel exhaust exposure was identified by reporting a history of work as a truck driver or as a heavy machinery operator.

The odds ratio for "possible exposure" to diesel exhaust is 1.47 (95% CI = 1.01, 2.13). When the criteria for exposure were "substantial" (defined by rating both Concentration and frequency of exposure as medium or high), the odds ratio is 1.10 (95% CI= 0.72, 1.67). Diesel exhaust showed an increase in risk with duration of exposure (1-10 vs. 11+ years). The odds ratio for 11 or more years of exposure is 1.5 (95% CI = 1.1, 2.1). Risk is not positively correlated with concentration (low vs. medium/high) or frequency of exposure (low/medium vs. high).

This study identifies associations between diesel exhaust exposure as inferred by occupational history and histologically confirmed incident prostate cancers. The crude exposure assessment and lack of a substantive a priori hypothesis for the relation require that the results be considered preliminary or exploratory. The lack of control for smoking and diminished effect with increasing certainty of exposure also undermine the credibility of the observed association.

7.2.4. Summaries of Studies and Meta-Analyses of Lung Cancer

7.2.4.1. *Cohen and Higgins (1995): Health Effects of Diesel Exhaust: Epidemiology*

The Health Effects Institute (HEI) reviewed all published epidemiologic studies on the health effects of exposure to diesel exhaust available through June 1993 identified by a MEDLINE search and by reviewing the reference sections of published research and earlier reviews. HEI identified 35 reports of epidemiologic studies (16 cohort and 19 case-control) of the relation of occupational exposure to diesel emissions and lung cancer published between 1957 and 1993.

HEI reviewed the 35 reports for epidemiologic evidence of health effects of exposure to diesel exhaust for lung cancer, other cancers, and nonmalignant respiratory disease. They found that the data were strongest for lung cancer. The evidence suggested that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding caused by cigarette smoking or other known sources of bias.

Control for smoking was identified in 15 studies. Six studies (17%) reported relative risk estimates less than one; 29 studies (83%) reported at least relative risk indicating positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals, which excluded unity.

The authors conclude that epidemiologic data consistently show weak associations between exposure to diesel exhaust and lung cancer. They find that the evidence suggests that long-term exposure to diesel exhaust in a variety of occupational circumstances is associated with a 1.2- to 1.5-fold increase in the relative risk of lung cancer compared with workers classified as unexposed. Most of the studies that controlled for smoking found that the association between increased risk of lung cancer and exposure to diesel exhaust persisted after such controls were applied, although in some cases the excess risk was lower. None of the studies measured exposure to diesel emissions or characterized the actual emissions from the source of exposure for the time period most relevant to the development of lung cancer. Most investigators classified exposure on the basis of work histories reported by subjects or their next of kin, or by retirement records. Although these data provide relative rankings of exposure, the absence of concurrent exposure information is the key factor that limits interpretation of the epidemiologic findings and subsequently their utility in making quantitative estimates of cancer risks.

This is a comprehensive and thorough narrative review of studies of the health effects of diesel exhaust. It does not undertake formal estimation of summary measures of effect or evaluation of heterogeneity in the results. The conclusion drawn about the consistency of the results is based on the author's assessment of the failure of potential biases and alternative explanations for the increase in risk to account for the observed consistency. In many if not most studies, the quality of the data used to control confounding was relatively crude. Although the studies do include qualitative assessment of whether control for smoking is taken into account, careful scrutiny of the quality of the control or adjustment for smoking among the studies is absent. This leaves open the possibility that prevalent residual confounding by inadequate control for smoking in many or most studies may account for the consistent associations seen.

7.2.4.2. *Bhatia et al. (1998): Diesel Exhaust Exposure and Lung Cancer*

Bhatia et al. (1998) report a meta-analysis of 29 published cohort and case-control studies of the relation between occupational exposure to diesel exhaust and lung cancer. A search of the epidemiologic literature was conducted for all studies concerning lung cancer and diesel exhaust exposure. Occupational studies involving mining were excluded because of concern about the possible influence of radon and silica exposures. Studies in which the minimum interval from time of first exposure to end of followup was less than 10 years, and studies in which work with diesel equipment or engines could not be confirmed or reliably inferred, were excluded. When studies presented risk estimates for more than one specific occupational category of diesel

exhaust-exposed workers, the subgroup risk estimates were used in the meta-analysis. Smoking-adjusted effect measures were used when present.

Thirty-five studies were identified in the literature search, of which 23 met the criteria for inclusion in the meta-analysis. The observed relative risk estimates were greater than 1 in 21 of these studies; this result is unlikely to be due to chance. The pooled relative risk weighted by study precision was 1.33 (95% CI = 1.24, 1.44) indicated increased relative risk for lung cancer from occupational exposure to diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons. (See Figure 7-1.)

Bhatia and colleagues conclude that the analysis shows a small but consistent increase in the risk for lung cancer among workers with exposure to diesel exhaust. The authors evaluate the dependence of the relative risk estimate on the presence of control for smoking among studies, and provide a table that allows assessment of whether the quality of the data contributing to control for smoking is related to the relative risk estimates (albeit in a limited number of studies). Bhatia et al. assert that residual confounding is not affecting the summary estimates or conclusions for the following reasons: (1) the pooled relative risks for studies adjusted for smoking were the same as those for studies not adjusting for smoking; (2) in those studies giving risk estimates adjusted for smoking and risk estimates not adjusted for smoking, there was only a small reduction in the pooled relative risk from diesel exhaust exposure; and (3) in studies with internal comparison populations, in which confounding is less likely, the pooled relative risk estimate was 1.43.

The validity of this assessment depends on the adequacy of control for smoking in the individual studies. If inadequate adjustment for smoking is employed and residual confounding by cigarette smoking pertains in the result of the individual studies, then the comparisons and contrasts of the pooled estimates they cite as reasons for dismissing the effect of residual confounding by smoking will remain contaminated by residual confounding in the individual studies. In fact, Bhatia et al. erroneously identify the treatment of the smoking data in the main analysis for the 1987 report by Garshick et al. as a continuous variable representing pack-years of smoking, whereas the analysis actually dichotomized the pack-years data into two crude dose categories (above and below the 50 pack-years level). This clearly reduced the quality of the adjustment for smoking, which already suffered from the fact that information on cumulative cigarette consumption was missing for more than 20% of the lung cancer cases. In this instance, the consistency between the adjusted and unadjusted estimates of the relative risk for diesel exhaust exposure may be attributable to failure of adjustment rather than lack of confounding by

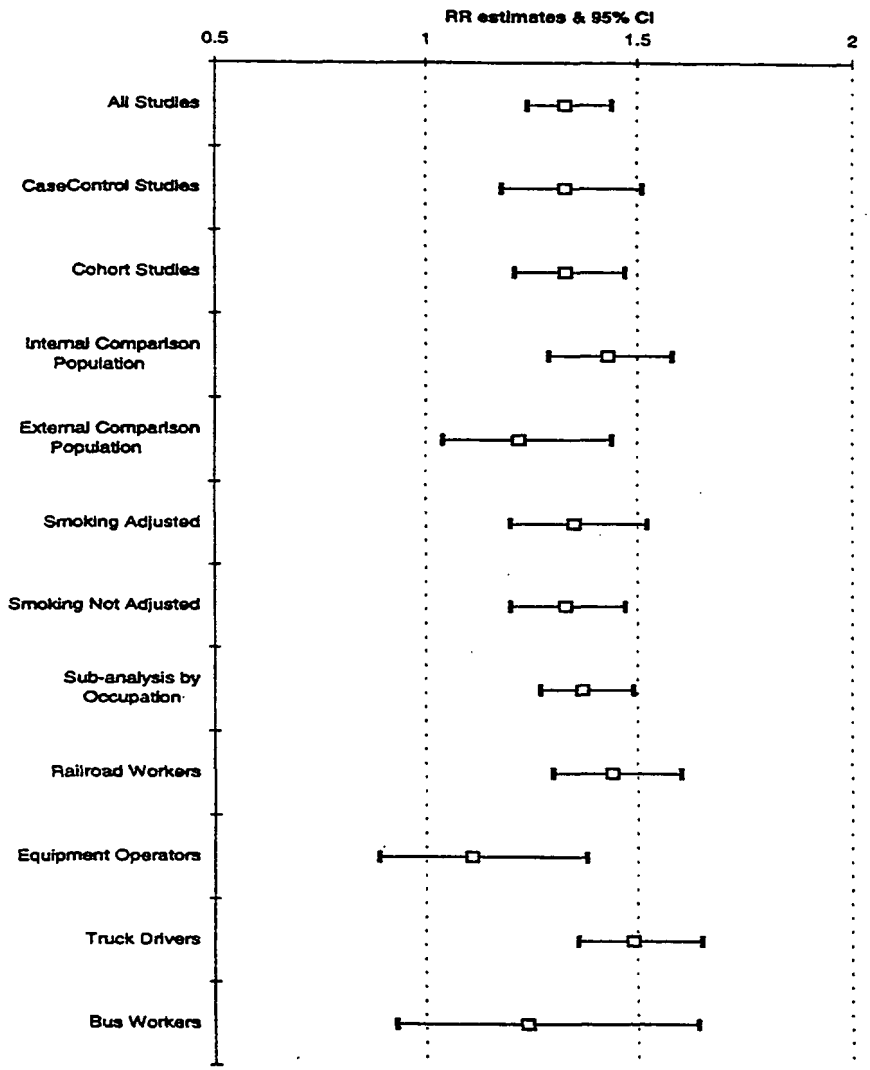


Figure 7-1. Pooled relative risk estimates and heterogeneity-adjusted 95% confidence intervals for all studies and subgroups of studies included in the meta-analysis.
Source: Bhatia et al., 1998.

cigarette smoking; and pooled estimates of association of diesel exhaust with lung cancer derived in the meta-analysis would remain confounded. A similar problem exists for the Bhatia et al. representation of the control for confounding in the study by Boffetta and Stellman (1988). Such mischaracterizations may indicate an overstatement by Bhatia et al. that the association of DE and lung cancer is insensitive to adjustment.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility for publication bias.

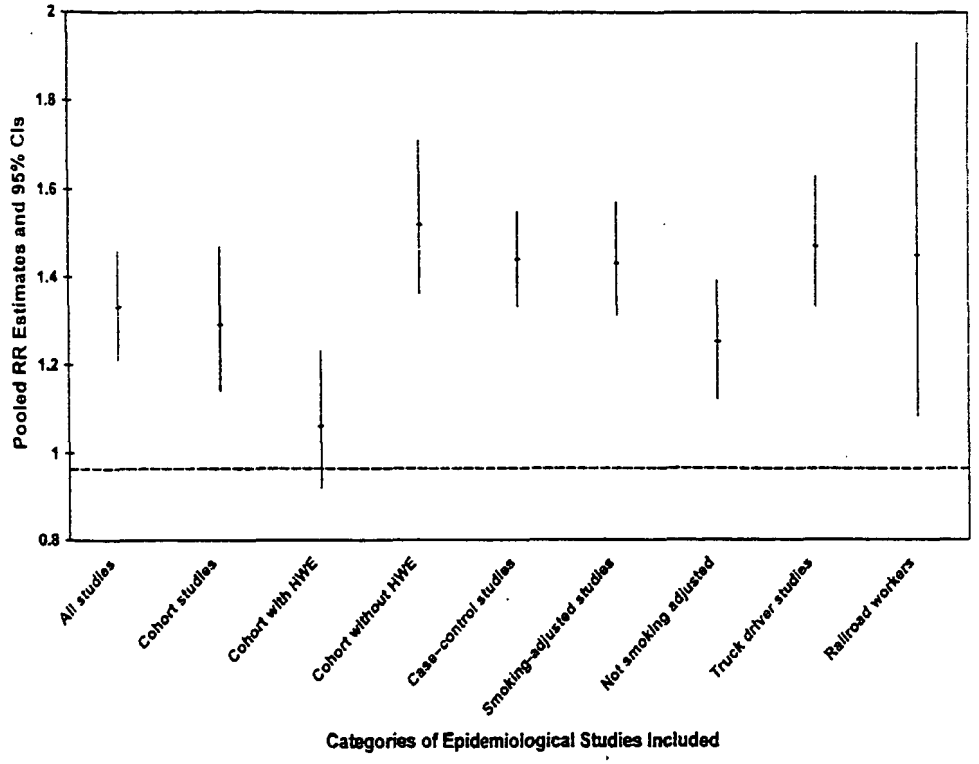
7.2.4.3. *Lipsett and Campleman (1999): Occupational Exposure to Diesel Exhaust and Lung Cancer: A Meta-Analysis*

Lipsett and Campleman (1999) conducted electronic searches to identify epidemiologic studies published between 1975 and 1995 of the relationship of occupational exposure to diesel exhaust and lung cancer. Studies were selected based on the following criteria. (1) Estimates of relative risks and their standard errors must be reported or derivable from the information presented. (2) Studies must have allowed for a latency period of 10 or more years for development of lung cancer after onset of exposure. (3) No obvious bias resulted from incomplete case ascertainment in followup studies. (4) Studies must be independent: that is, a single representative study selected from any set of multiple analyses of data from the same population. Studies focusing on occupations involving mining were excluded because of potential confounding by radon, arsenic, and silica, as well as possible interactions between cigarette smoking and exposure to these substances in lung cancer induction.

Thirty of the 47 studies initially identified as relevant met the specified inclusion criteria. Several risk estimates were extracted from six studies reporting results from multiple mutually exclusive diesel-related occupational subgroups. If a study reported effects associated with several levels or durations of exposure, the effect reported for the highest level or longest duration of exposure was used. If estimates for several occupational subsets were reported, the most diesel-specific occupation or exposure was selected. Adjusted risk estimates were used when available.

Thirty-nine independent estimates of relative risk and standard errors were extracted. Pooled estimates of relative risk were calculated using a random-effects model. Among study populations most likely to have had substantial exposure to diesel exhaust, the pooled smoking adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). (See Figure 7-2.)

The between-study variance of the relative risks indicated the presence of significant heterogeneity in the individual estimates. The authors evaluated the potential sources of



Note. CI = confidence interval; HWE = healthy worker effect.

Figure 7-2. Pooled estimates of relative risk of lung cancer in epidemiological studies involving occupational exposure to diesel exhaust (random-effects models).

Source: Lipsett and Campleman, 1999.

heterogeneity by subset analysis and linear metaregressions. Major sources of heterogeneity included control for confounding by smoking, selection bias (a healthy worker effect), and exposure patterns characteristic of different occupational categories. A modestly higher, pooled relative risk was derived for the subset of case-control studies, which, unlike the cohort studies, showed little evidence of heterogeneity.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility of publication bias.

Although a relatively technical approach was used in deriving summary estimates of relative risk and the evaluation of possible sources of variation in the relative risks in this meta-analysis, this approach should not be confused with rigorous evaluation of the potential weaknesses among the studies included in the analysis. The heterogeneity attributable to statistical adjustment for smoking was evaluated based on a dichotomous assessment of whether control for smoking could be identified in the studies considered. This does not reflect the adequacy of the adjustment for smoking employed in the individual studies considered. The potential for residual confounding by inadequate adjustment for the influence of smoking remains in the summary estimate of the relative risk.

7.2.5. Case-Control Studies of Bladder Cancer

7.2.5.1. *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 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, and so on. 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.

7.2.5.2. *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 before 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 with 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.

7.2.5.3. *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 control was selected with the additional matching criterion 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.

7.2.5.4. *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.

7.2.5.5. *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.

7.2.5.6. *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.

7.2.5.7. *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 7-3 summarizes the bladder cancer case-control studies.

7.2.6. Discussion and Summary

Certain extracts of diesel exhaust have been demonstrated as 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

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer

Authors	Population studied	Diesel exhaust exposure	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	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 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

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	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 ($p \leq 0.05$) 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

Table 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

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
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	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 7-3. Epidemiologic studies of the health effects of exposure to diesel exhaust: case-control studies of bladder cancer (continued)

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.

to this complex mixture may be carcinogenic to humans has become an important public health issue.

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 is 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 measurement of actual diesel exhaust exposure. Because all the cohort mortality studies were retrospective, 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 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). The investigators found 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. Quantitative use of this information would have been much more meaningful. 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), no known studies of miners have assessed 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 and 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 or heavy metals (e.g., arsenic, chromium). Furthermore, the relatively short latency period limits the usefulness of these cohorts of miners.

7.2.6.1. *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 followup 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 followup 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 significantly 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, followup 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 followup 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.

7.2.6.2. *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. Furthermore, 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 dockworkers 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.

7.2.6.3. *Reviews and Meta-analyses of Lung Cancer*

Three summaries of studies concerned with the relationship of diesel exhaust exposure and lung cancer risk are reviewed. The HEI report is a narrative study of more than 35 epidemiologic studies (16 cohort and 19 case-control) of occupational exposure to diesel emissions published between 1957 and 1993. Control for smoking was identified in 15 studies. Six of the studies (17%) reported relative risk estimates less than 1, whereas 29 (83%) reported at least 1 relative risk, indicating a positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals that excluded unity. These studies found that the evidence suggests that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding due to cigarette smoking of other known sources of bias.

Bhatia et al. (1998) identified 23 studies that met criteria for inclusion in the meta-analysis. The observed relative risk estimates were greater than 1 in 21 of these studies. The pooled relative risk weighted by study precision was 1.33 (95% CI= 1.24, 1.44), which indicated increased relative risk for lung cancer from occupational exposure to diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons.

Lipsett and Campleman (1999) identify 39 independent estimates of relative risk among 30 eligible studies of diesel exhaust and lung cancer published between 1975 and 1995. Pooled relative risks for all studies and for study subsets were estimated using a random effect model. Interstudy heterogeneity was also modeled and evaluated. A pooled smoking-adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). Substantial heterogeneity was found in the pooled-risk estimates. Adjustment for confounding by smoking, having a lower likelihood of selection bias, and increased study power were all found to contribute to lower heterogeneity and increased pooled estimates of relative risk.

There is some variability in the conclusions of these summaries of the association of diesel exhaust and lung cancer. The three analyses find that smoking is unlikely to account for the observed effects, and all conclude that the data support a causal association between lung cancer and diesel exhaust exposure. On the other hand, Stober and Abel (1996), Muscat and

Wynder (1995) and Cox (1997) call into question the assertions by Cohen and Higgins (1995), Bhatia et al. (1997), and Lipsett and Campleman (1999) that the associations seen for diesel exhaust and lung cancer are unlikely to be due to bias. They argue that methodologic problems are prevalent among the studies, especially in evaluation of diesel engine exposure and control of confounding by cigarette smoking. The conclusions of the two meta-analyses are based on magnitude of pooled relative risk estimates and evaluation of potential sources of heterogeneity in the estimates. Despite the statistical sophistication of the meta-analyses, the statistical models used cannot compensate for deficiencies in the original studies and will remain biased to the extent that bias exists in the original studies.

It should be noted that a recent publication by Bruske-Hohlfeld et al. (1999) found a strong association between DE exposure and the occurrence of lung cancer. This pooled analysis of two case-control studies has a large sample size, is adjusted for smoking and asbestos exposures, and exposure to DE was estimated on the basis of job codes. This study is not critiqued in detail here but will be included when the document is finalized.

7.2.6.4. *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.

7.2.6.5. *Relevant Methodologic Issues*

Throughout this chapter, various methodologic limitations of individual studies have been discussed, such as small sample size, short followup 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 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 cohort mortality studies and some of the case-control studies of lung cancer and case-control studies 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.

A persistent association of risk for lung cancer and diesel exhaust exposure is observed in more than 30 epidemiologic studies published over the past 40 years. Evaluation of whether this association can be attributed to a causal relation between diesel exhaust exposure and lung cancer requires careful consideration of whether chance, bias, or confounding might be likely alternative explanations.

Many of the studies provide confidence intervals for their estimates of excess risk or statistical tests, which indicate that it is unlikely that the individual study findings were due to random variation. The persistence of this association between diesel exhaust and lung cancer risk in so many studies indicates that the possibility is remote that the observed association in aggregate is due to chance. It is unlikely that chance alone accounts for the observed relation between diesel exhaust and lung cancer.

The excess risk is observed in both cohort and case-control designs, which contradicts the concern that a methodologic bias specifically characteristic of either design (e.g., recall bias) might account for the observed effect. Selection bias is certainly present in some of the occupational cohort studies that use external population data in estimating relative risks, but this

form of selection bias (a healthy worker effect) would only obscure, rather than spuriously produce, an association between diesel exhaust and lung cancer. Several occupational epidemiologic studies that use more appropriate data for their estimates are available. Selection biases may be operating in some case-control studies, but it is not obvious how such a bias could be sufficiently uniform in effect, prevalent, and strong enough to lead to the persistent association seen in the aggregate data. Given the variety of designs used in studying the diesel exhaust and lung cancer association and the number of studies in different populations, it is unlikely that routinely studying noncomparable groups is an explanation for the persistent association seen. Exposure information bias is certainly a problem for almost all of the studies concerned. Detailed and reliable individual-level data on diesel exhaust exposure for the period of time relevant to the induction of lung cancer are not available and are difficult to obtain. Generally, the only information from which diesel exposure can be inferred is occupational data, which is a poor surrogate for the true underlying exposure distribution. Study endpoints are frequently mortality data taken from death certificate information, which is frequently inaccurate and often does not fully characterize the lung cancer incidence experience of the population in question. Using inaccurate surrogates for lung cancer incidence and for diesel exposure can lead to substantial bias, and these shortcomings are endemic in the field. In most cases these shortcomings will lead to misclassification of exposure and of outcome, which is nondifferential. Nondifferential misclassification of exposure and/or outcome can bias estimates of a diesel exhaust–lung cancer association, if one exists, toward the null; but it is unlikely that such misclassification would produce a spurious estimate in any one study. It is even more unlikely that it would bias a sufficient number of studies in a uniform direction to account for the persistent aggregate association observed.

All the cohort studies considered for this report are retrospective mortality studies. Smoking history is usually difficult to obtain in such instances. The smoking histories obtained from surrogates (next of kin, either spouse or offspring) were found to be accurate by Lerchen and Samet (1986) and McLaughlin et al. (1987). Lerchen and Samet 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. found that the age at which an individual started smoking was reported within 4 years of actual age 84% of the time. These studies indicate 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 compared with 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.

Many studies do not adjust for tobacco smoke exposure. These studies are correctly dismissed as not contributing to the body of information suitable for causal inference. Several studies do attempt to adjust for smoking. Sometimes the data are aggregate data and the methods used for adjustment are indirect and rely on critical and unverifiable assumptions for effective adjustment (Pfluger, 1994). Frequently, individual-level data are used to adjust estimates of effect by conventional methods. Usually, these data are not a careful, detailed, and thorough assessment of smoking behavior. Generally the classification of smoking behavior is crude (smoker vs. nonsmoker) and cannot be considered to fully characterize actual exposure. Given these shortcomings, a possibility remains that the statistical adjustment for smoking is not completely effective, and residual confounding by smoking may persist to bias the measure of the diesel exhaust-lung cancer association.

7.2.6.6. *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, this 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 in every cohort and case-control study.

- **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 with 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 compared with 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 7-3 and 7-4). Meta-analyses by Bhatia et al. and Lipsett et al. also show the pooled estimated RR of 1.33 and 1.47, respectively. 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.

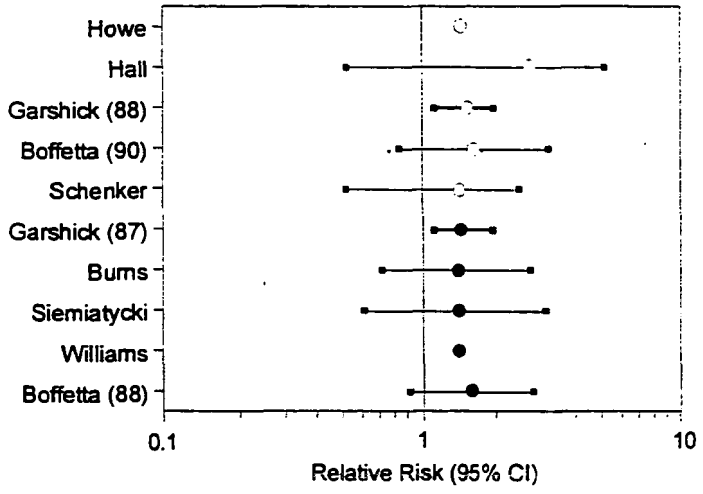


Figure 7-3. Lung cancer and exposure to diesel exhaust in railroad workers. ● = Relative risk adjusted for cigarette smoking; ○ = relative risk not adjusted for cigarette smoking. For the two studies by Howe and Williams, confidence intervals were not reported and could not be calculated.

Source: HEI, 1995.

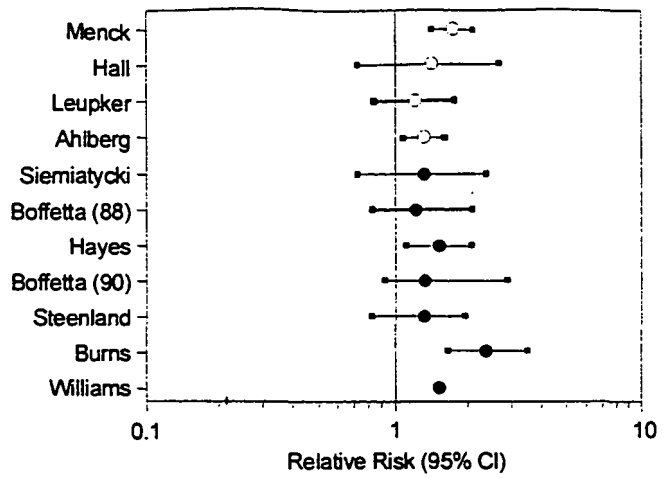


Figure 7-4. Lung cancer and exposure to diesel exhaust in truck drivers. ● = Relative risk adjusted for cigarette smoking; O = relative risk not adjusted for cigarette smoking. For the study by Williams, confidence intervals were not reported and could not be calulated. For the Steenland study, the data 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).

urce: HEI, 1995.

- **Consistency:** Several cohort and case-control (including one nested 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 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.

- **Strength of association:** The relative odds of getting bladder cancer among exposed compared with 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, or urinary retention.

- **Consistency:** Four out of seven bladder case-control studies conducted in the United States and abroad found 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 several cohort and case-control studies. A recent meta-analysis shows the consistency of elevated risks in 23 of 29 diesel exposure epidemiologic studies, with statistically significant relative risks of 1.33 (Bhatia et al., 1998). Lipsett et al. (1999) also found a pooled estimate RR of 1.47 after adjusting for smoking. 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.

7.3. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. The first portion of this chapter summarizes results of inhalation studies. Experimental protocols for the inhalation studies typically 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. Other studies were designed to evaluate the relative importance of the carbon core of the diesel particle versus that of particle-adsorbed compounds. Finally, a number of exposures were carried out to determine the combined effect of inhaled diesel exhaust and tumor initiators, tumor promoters, or co-carcinogens.

Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 mg/m^3. 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 inhalation carcinogenicity studies and their results is presented in Table 7-4.

Results of lung implantation and intratracheal instillation studies of whole diesel particles, extracted diesel particles, and particle extracts are reported in Section 7.3.3 and in Tables 7-6 and 7-7. Studies destined to assess the carcinogenic effects of DPM as well as solvent extracts of DPM following subcutaneous (s.c.) injection, intraperitoneal (i.p.) injection, or intratracheal (itr.) instillation in rodents are summarized in Section 7.3.5. Individual chemicals present in the gaseous phase or adsorbed to the particle surface were not included in this review because assessments of those of likely concern (i.e., formaldehyde, acetaldehyde, benzene, PAHs) have been published elsewhere (U.S. EPA, 1993).

7.3.1. Inhalation Studies (Whole Diesel Exhaust)

7.3.1.1. *Rat Studies*

The potential carcinogenicity of inhaled diesel exhaust was first evaluated by Karagianes et al. (1981). Male Wistar rats (40 per group) were exposed to room air or diesel engine exhaust diluted to a DPM concentration of 8.3 (± 2.0) mg/m^3, 6 hr/day, 5 days/week for up to 20 mo. The animals were exposed in 3,000 liter plexiglass chambers. Airflow was equal to 50 liters per minute. Chamber temperatures were maintained between 25° and 26.5 °C. Relative humidity ranged form 45% to 80%. Exposures were carried out during the daytime. 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

Table 7-4. Summary of animal inhalation carcinogenicity studies

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]	Comments
								Adenomas	
Karagianes et al. (1981)	Rat/ Wistar	M, 40	Clean air	0	None	6 hr/day, 5 days/week, for up to 20 mo	NA	0/6 (0)	
		M, 40	Whole exhaust	8.3	None			1/6 (16.6)	
								Broncho-alveolar carcinoma	
Kaplan et al. (1983) White et al. (1983)	Rat/F344	M, 30	Clean air	0	None	20 hr/day, 7 days/week, for up to 15 mo	8 mo	0/30 (0)	
		M, 30	Whole exhaust	0.25	None		8 mo	1/30 (3.3)	
		M, 30	Whole exhaust	0.75	None		8 mo	3/30 (10.0)	
		M, 30	Whole exhaust	1.5	None		8 mo	1/30 (3.3)	
								Adenomas / Carcinomas / Squamous cell tumors / All tumors	
Heinrich et al. (1986a,b) Mohr et al. (1986)	Rat/ Wistar	F, 96	Clean air	0	None	19 hr/day, 5 days/week for up to 35 mo	NA	0/96 (0) / 0/96 (0) / 0/96 (0) / 0/96 (0)	
		F, 92	Filtered exhaust	0	None			0/92 (0) / 0/92 (0) / 0/92 (0) / 0/92 (0)	
		F, 95	Whole exhaust	4.0	None			8/95 (8.4) / 0/95 (0) / 9/95 (9.4) / 17/95 (17.8)[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 hr/day, 7 days/week, for 24 mo	NA	1/22 (4.5) / 0/22 (0) / 0/22 (0) / 1/22 (4.5)[c]	
		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,d]	
								Adenoma / Carcinoma	
Takemoto et al. (1986)	Rat/F344	F, 12	Clean air	0	None	4 hr/day, 4 days/week, 18-24 mo	NA	0/12 (0) / 0/12 (0)	
		F, 21	Clean air	0	DIPN[b]			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[b]			12/18 (66.7) / 7/18 (38.9)	
								Adenomas / Adenocarcinoma + squamous cell carcinoma / Squamous cysts / All tumors	
Mauderly et al. (1987)	Rat/F344	M + F, 230[b]	Clean air	0	None	7 hr/day, 5 days/week up to 30 mo	NA	(0) / (0.9) / (0) / (0.9)	
		M + F, 223	Whole exhaust	0.35	None			(0) / (1.3) / (0) / (1.3)	
		M + F, 221	Whole exhaust	3.5	None			(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]	

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenosquamous carcinomas	Squamous cell carcinomas	All tumors	
Ishinishi et al. (1988a)	Rat/F344	M + F, 123	Clean air	0	None	16 hr/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)	
Heavy-duty engine		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	Carcinomas	All tumors		
Ishinishi et al. (1988a)	Rat/F344	NS, 5	Whole exhaust	0.1	None	16 hr/day, 6 days/week, for 12 mo	6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 8	Whole exhaust	0.1	None		12 mo	0/8 (0)	0/8 (0)	0/8 (0)		
		NS, 11	Whole exhaust	0.1	None		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 hr/day, 6 days/week, for 12 mo	6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 9	Whole exhaust	0.5	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)		
		NS 11	Whole exhaust	0.5	None		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)		
								Primary lung tumors				
Brightwell et al. (1989)	Rat/344	M + F, 260	Clean air	0	None	16 hr/day, 5 days/week, for 24 mo	NA		3/260 (1.2)			
		M + F, 144	Filtered exhaust (medium exposure)	0	None				0/144 (0)			Tumor incidence for all rats dying or sacrificed
		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]			

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure 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 hr/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)	
Lewis et al. (1989)	Rat/F344	M + F, 288[a]	Clean air	0	None	7 hr/day, 5 days/week, 24 mo	NA	No tumors			0/192 (0)	
			Whole exhaust	2.0	None						0/192 (0)	
								Adenosquamous carcinomas	Squamous cell carcinomas	All tumors		
Takaki et al. (1989) Light-duty engine	Rat/F344	M + F, 123	Clean air	0	None	16 hr/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	Adenocarcinomas	Squamous cell carcinomas	Benign squamous cell tumors	
Heinrich et al. (1995)	Rat/ Wistar	F, 220	Clean air	0	None	18 hr/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
		F, 100	Carbon black	11.6	None			13/100 (13)	13/100 (13)	4/100 (4)	20/100 (20)	incidences
		F, 100	TiO_2	10.0	None			4/100 (4)	13/100 (13)	3/100 (3)	20/100 (20)	after 30 mo
								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 hr/day, 5 days/week for up to 24 mo	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			7/210 (3)	4/210 (2)	3/210 (1)	0/210 (0)	0/210 (0)
		M + F, 212	Whole exhaust	6.5	None			23/212 (11)	22/212 (10)	3/212 (1)	1/212 (<1)	0/212 (0)
		M + F, 213	Carbon black	2.5	None			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)
Iwai et al. (1997)	F/344	121, F	Clean air	0	None	NA	NA	5/121(4%) type not stated				Cumulative exposure dose ranged from 154-274 mg/cum
		108, F	Filtered air	0	None	48-56 hr/day	6 mo	2/108(4%) type not stated				
		153, F	Whole exhaust	3.2-9.4	None	48-56 hr/day	6 mo	53/153(35%) 61.3% adenoma, 25.8% adenocarcinoma, 2.2% benign squamous cell tumor, 7.5% squamous cell carcinoma, 3.2% adenosquamous carcinoma				

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]	Comments
Orthoefer et al. (1981) (Pepelko and Peirano, 1983)	Mouse/ Strong A	M, 25	Clean air	0	None	20 hr/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 hr/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 hr/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
Kaplan et al. (1982)	Mouse A/J	M, 458	Clean air	0	None	20 hr/day, 7 days/week, for 3 mo	6 mo	Pulmonary adenomas	
		M, 18	Clean air	0	Urethan[k]			144/458 (31.4)	
		M, 485	Whole exhaust	1.5	None			18/18 (100)	
								165/485 (34.2)	
								Pulmonary adenoma	

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]	Comments
Kaplan et al. (1983)	Mouse/ A/ J	M, 388	Clean air	0	None	20 hr/day,	NA	130/388 (33.5)	
		M, 388	Whole exhaust	0.25	None	7 days/week,		131/388 (33.8)	
White et al. (1983)		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)	

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	Adenomas	Carcinomas	All tumors	Comments
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[j]	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[j]			(5.4)	(2.7)	(8.1)	
			Whole exhaust	12	Urethan[j]			(8.7)	(2.6)	(11.2)	

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	All tumors	Comments
Pepelko and Peirano (1983)	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

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors	Comments
Heinrich et al. (1986a,b)	Mouse/ NMRI	M + F, 84	Clean air	0	None	19 hr/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]	

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	Adenoma	Adenocarcinoma	Comments
Takemoto et al. (1986)	Mouse/ IRC	M + F, 45	Clean air	0	None	4 hr/day,	NA			
		M + F, 69	Whole exhaust	2-4	None	4 days/week, for 19-28 mo				
	Mouse/ C57BL	M + F, 12	Clean air	0	None	4 hr/day,	NA	3/45 (6.7)	1/45 (2.2)	
		M + F, 38	Whole exhaust	2-4	None	4 days/week for 19-28 mo		6/69 (8.7)	3/69 (4.3)	

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m^3)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
Heinrich et al. (1995)	Mouse/ C57BL/	F, 120	Clean air	0	None	18 hr/day, 5 days/week, for up to 21 mo	6 mo	1/12 (8.3)	0/12 (0)			5.1% tumor rate
	6N	F, 120	Whole exhaust	4.5	None			8/38 (21.1)	3/38 (7.9)			8.5% tumor rate
		F, 120	Particle-free exhaust	0	None							3.5% tumor rate
	Mouse/ NMRI	F, 120	Clean air	0	None	18 hr/day, 5 days/week for up to 13.5 mo	9.5 mo	Adenomas	Adenocarcinomas			
		F, 120	Whole exhaust	4.5	None			(25)	(15.4)			
			Carbon black	11.6	None			(21.8)	(15.4)			
			TiO_2	10	None			(11.3)	(10)			
								(11.3)	(2.5)			
	Mouse/ NMRI	F, 20	Clean air	0	None	18 hr/day, 5 days/week, 23 mo	None	(25)	(8.8)			
		F, 20	Whole exhaust	4.5	None			(18.3)	(5.0)			
		F, 20	Particle-free exhaust	0	None			(31.7)	(15)			
								Multiple adenomas	Multiple carcinomas	Adenomas/ carcinoma	Alveolar/ bronchiolar adenoma	Alveolar/ bronchiolar carcinoma
Mauderly et al. (1996)	Mouse/ CD-1	M + F 157[b]	Clean air	0	None	7 hr/day, 5 days/week, for up to 24 mo	None	1/157 (0.6)	2/157 (1.3)	1/157 (0.6)	10/157 (6.4)	7/157 (4.5)
		M + F 171	Whole exhaust	0.35	None			2/171 (1.2)	1/171 (0.6)	1/171 (0.6)	16/171 (9.4)	5/171 (2.9)
		M + F 155	Whole exhaust	3.5	None			0/155 (0)	1/155 (0.6)	0/155 (0)	8/155 (5.2)	6/155 (3.9)
		M + F 186	Whole exhaust	7.0	None			0/186 (0)	0/186 (0)	0/186 (0)	10/186 (5.4)	4/186 (2.2)

Table 7-4. Summary of animal inhalation carcinogenicity studies (continued)

Study	Species/ strain	Sex/total number	Exposure atmosphere	Particle concentration (mg/m³)	Other treatment	Exposure protocol	Post-exposure observation	Tumor type and incidence (%)[a]				Comments
								Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b)	Hamster/ Syrian	M + F, 96	Clean air	0	None	19 hr/day		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	NA	0/96(0)	0/96(0)	0/96	0/96(0)	
									Primary lung tumors			
Brightwell et al. (1989)	Hamster/ Syrian Golden	M + F,	Clean air	0	None	16 hr/day,	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]	5 days/week,			4/104 (3.8)			
		M + F, 104	Filtered exhaust (medium dose)	0	DEN[j]	for 24 mo			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							

[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]Dipentylnitrosamine; 6.25 mg/kg/week s.c. during first 25 weeks of exposure.
[e]Dipentylnitrasamine; 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/diethylnitrosamine (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/m³from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 mg/m³.
[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.

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. Six rats per group were sacrificed after 4, 8, 16, and 20 mo exposure for gross necropsy and histopathological examination.

The only tumor detected was a bronchiolar adenoma in the group exposed over 16 mo to diesel exhaust. No lung tumors were reported in controls. The equivocal response may have been caused by the relatively short exposure durations (20 mo) and small numbers of animals examined. In more recent studies, for example, Mauderly et al. (1987), most of the tumors were detected in rats exposed for more than 24 mo.

General Motors Research Laboratories sponsored chronic inhalation studies at the Southwest Research Institute using male Fischer 344 rats, 30 per group, exposed to DPM concentrations of 0.25, 0.75, or 1.5 mg/m^3 (Kaplan et al., 1983; White et al., 1983). The animals were exposed in 12.6 m^3 exposure chambers. Airflow was adjusted to provide 13 changes per hour. Temperature was maintained at 22 ± 2 °C. The exposure protocol was 20 hr/day, 7 days/week for 9 to 15 mo. Exposures were halted during normal working hours for servicing. 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.

Although 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, compared with none among controls, statistical significance was not achieved in any of the exposure groups. 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. Equivocal results may again have been due to less-than-lifetime duration of the study as well as insufficient exposure concentrations. 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, relative to controls, they were slightly greater than the historic background incidence of 3.7% for this specific lesion in this strain of rat (Ward, 1983). The first definitive studies linking inhaled diesel exhaust to induction of lung cancer in rats were reported by researchers in Germany, Switzerland, Japan, and the United States in the mid-to-late 1980s. In a study conducted at the Fraunhofer Institute of Toxicology and Aerosol Research, female Wistar rats were exposed for 19 hr/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. (1986a) used a 40 kilowatt 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 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. (1986b) and Stöber (1986). Exposure to unfiltered exhaust resulted in 8 bronchoalveolar adenomas and 9 squamous cell tumors in 15 of 95 female Wistar 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 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,b) 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.

Iwai et al. (1986) 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 hr/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. Nineteen of the 24 exposed to unfiltered exhaust survived for 2 years. Of these, 14 were randomly selected for sacrifice at this time. Four of the rats 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 7-4. 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 was conducted by Takemoto et al. (1986), in which female Fischer 344 rats were exposed to diesel exhaust generated by a 269-cc YANMAR-40CE NSA engine operated at an idle state (1,600 rpm). Exposures were 4 hours/day, 4 days/week. The animals were exposed in a 376-L exposure chamber. Air flow was maintained at 120 L/min. Exhaust was diluted to produce a particle concentration of 2-4 mg/m^3. Concentrations of the gas-phase components of the exhaust are presented in Appendix A. When not exposed the animals were maintained in an air-conditioned room at a temperature of 24 ± 2°C and a relative humidity of 55 ± 5% with 12 hr of light and darkness. Temperature and humidity in the exposure chambers was not noted. 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. No lung tumors were reported in the diesel-exposed animals. 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 effects of diesel emissions.

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 hr/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%, respectively). In the high-dose group the incidences of tumor types reported were adenoma (0.4%), adenocarcinomas plus squamous cell carcinomas (7.5%), and squamous cysts (4.9%). In the medium-dose group adenomas were reported in 2.3% of animals, adenocarcinomas plus squamous cell carcinomas in 0.5%, and squamous cysts in 0.9%. In the low-exposure group adenocarcinomas plus squamous cell carcinomas were detected in 1.3% of the rats. 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 7-4. 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 long-term inhalation study (Ishinishi et al., 1988a; Takaki et al., 1989) examined the effects of emissions from a light-duty (LD) and a heavy-duty (HD) diesel engine 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 1,700 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. 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. The carcinomas were identified as adenomas, adenosquamous carcinomas, and squamous cell carcinomas. Although the number of each was not reported, it was noted that the

majority were squamous cell carcinomas. A progressive dose-response relationship was not demonstrated. Tumor incidence data for this experiment are presented in Table 7-4.

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.

Brightwell et al. (1986, 1989) studied the effects of 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 was replaced after 15 mo. 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. The CO and NO_x concentrations (mean ± SD) were 32 ± 11 ppm and 8 ± 1 ppm in the high-exposure concentration chamber. 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 1.2% (3/260), 0.7% (1/144), 9.7% (14/144), and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to clean air or 0.7, 2.2, and 6.6 mg of DPM/m^3, respectively (Table 7-4). Diesel exhaust-induced tumor incidence in rats was dose-related and higher in females than in males (Table 7-4). 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 numbers and types of tumors identified in the rats exposed to diesel exhaust included adenomas (40), squamous cell carcinomas (35), adenocarcinomas (19), mixed adenoma/adenocarcinomas (9), and mesothelioma (1). It should be noted that 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.

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 during daylight hours 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. The animals were exposed in 100-cubic-foot chambers. Temperature was controlled at 22±2 °C and relative humidity at 50±10%. The exhaust was diluted 27-fold with chemically and biologically filtered clean air to achieve the desired particle concentration. 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.

In a more recent study reported by Heinrich et al. (1995), 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, then held in clean air an additional 6 mo. The animals were exposed in either 6 or 12 m^3 exposure chambers. Temperature and relative humidity were maintined at 23-25 °C and 50%-70%, repectively. Diesel exhaust was generated by two 40-kw 1.6-L diesel engines (Volkswagen). One of them was operated according to the U.S. 72 cycle. The other was operated under constant load conditions. The first engine did not supply sufficient exhaust, which was filled by the second engine. Cumulative exposures for the rats in the various treatment groups were 61.7, 21.8, and 7.4 g/m^3 × h for the high, medium, and low whole-exhaust exposures. Significant increases in tumor incidences 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 7-4). 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.

Particle lung burden and alveolar clearance also were determined in the Heinrich et al. (1995) study. Relative to clean air controls, alveolar clearance was significantly compromised by

exposure to mid and high diesel exhaust. For the high-diesel-exhaust group, 3-mo recovery time in clean air failed to reverse the compromised alveolar clearance.

In a study conducted at the Inhalation Toxicology Research Institute (Nikula et al., 1995) F344 rats (114-115 per sex per group) were exposed 16 hr/day, 5 days/week during daylight hours to diesel exhaust diluted to achieve 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. Surviving rats were maintained an additional 6 weeks in clean air, at which time mortality reached 90%. Diesel exhaust was generated using two 1988 Model LH6 General Motors 6.2-L V-8 engines burning D-2 fuel that met EPA certification standards. Chamber air flow was sufficient to provide about 15 exchanges per hour. Relative humidity was 40% to 70% and temperature ranged from 23 to 25 °C.

Following 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. The percentages of susceptible rats (males and females combined) with malignant neoplasms were 0.9 (control), 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), 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. Among 212 males and females examined in the high-dose group, adenomas were detected in 23 animals, adenocarcinomas in 22 animals, squamous cell carcinomas in 3 animals, and an adenosquamous carcinoma in 1 animal. For further details see Table 7-4. Analysis of the histopathologic data suggested a progressive process from alveolar epithelial hyperplasia to adenomas and adenocarcinomas.

Iwai et al. (1997) carried out a series of exposures to both filtered and whole exhaust using a light-duty (2,369 mL) diesel engine. The protocol for engine operation was not stated. Groups of female SPF F344 Fischer rats were exposed for 2 years for 8 hr/day, 7 days/week, 8 hr/day, 6 days/week, or 18 hr/day, 3 days/week to either filtered exhaust or exhaust diluted to a particle concentration of 9.4, 3.2, and 5.1 mg/m^3, respectively. Cumulative exposure (mg/m^3 × hrs of exposure) equaled 274.4, 153.6, and 258.1 mg/m^3. The animals were then held for an additional 6 mo in clean air. Lung tumors were reported in 5/121 (4%) of controls, 4/108 (4%) of those exposed to filtered exhaust, and 50/153 (35%) among those exposed to whole exhaust. Among rats exposed to whole diesel exhaust the following number of tumors were detected; 57 adenomas, 24 adenocarcinomas, 2 benign squamous cell tumors, 7 squamous cell carcinomas, and 3 adenosquamous carcinomas. The authors stated that benign squamous cell tumors probably corresponded to squamous cysts in another classification.

7.3.1.2. *Mouse Studies*

A series of inhalation studies using strain A mice was conducted by Orthoefer et al. (1981). Strain A mice are usually given a series of intraperitoneal injections with the test agent; they are then sacrificed at about 9 mo and examined for lung tumors. In the present series, inhalation exposure was substituted. Diesel exhaust was provided by one of two Nissan CN6-33 diesel engines having a displacement of 3244 cc and run on a Federal Short Cycle. Flow through the exposure chambers was sufficient to provide 15 air changes per hour. Temperature was maintained at 24 °C and relative humidity at 75%. In the first study, groups of 25 male Strong A strain (A/S) mice were exposed to irradiated diesel exhaust (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m^3) for 20 h/day, 7 days/week. Additional groups of 40 Jackson A strain (S/J) mice (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/S mice were exposed 8 hr/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 7-4.

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 in inhalation chambers to diesel exhaust generated by a 5.7-L Oldsmobile engine operated continuously at 40 mph at DPM concentrations of 0, 0.25, 0.75, or 1.5 mg/m^3. Controls were exposed to clean air. Temperture was maintained at 22 ± 2 °C and relative humidity at 50 ± 10% within the chambers. 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 7-4).

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 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-hr/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. Chamber temperature was maintained at 24 °C and relative humidity at 50%. Airflow was sufficient for 15 changes per hour.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust. 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) were exposed to either diesel exhaust or clean air. 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 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. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The 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 likely that Sencar mice are sensitive to induction of lung tumors since they are also sensitive to induction of skin tumors. These data are summarized in Table 7-4.

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 Section 7.3.2.1 and Appendix A. All numbers reported are for males and females combined. Four adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-exposed ICR mice autopsied at 13 to 18 mo, compared with 3 adenomas among 38 controls. Six adenomas and 3 adenocarcinomas were reported in 22 diesel-exposed ICR mice autopsied at 19 to 28 mo, compared with 3 adenomas and 1 adenocarcinoma in 22 controls. Four adenomas and 2 adenocarcinomas were detected in 79 C57BL mice autopsied at 13 to 18 mo, 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

were reported for either strain of exposed mice. However, the significance of the increase in the combined incidence of adenomas and carcinomas was not evaluated statistically. A statistical analysis by Pott and Heinrich (1990) indicated that the difference in combined benign and malignant tumors between whole diesel exhaust-exposed C57BL/6N mice and corresponding controls was significant at $p<.05$. See Table 7-4 for details of tumor incidence.

Heinrich et al. (1986b) 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. (1986a) are given in Section 7.3.1.1 and Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to diesel exhaust diluted to achieve a particle concentration of 4.2 mg/m^3, 76 female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 32%. Tumor incidences reported for control mice (n = 84) equaled 11% for adenomas and adenocarcinomas combined. While the incidence of adenomas showed little change, adenocarcinomas increased significantly from 2.4% for controls to 17% for exhaust-exposed mice. In a follow up study, however, Heinrich et al. (1995) reported a lack of tumorigenic response in either female NMRI or C57BL/6N mice exposed 17 h/day, 5 days/week for 13.5 to 23 mo to whole diesel exhaust diluted to produce a particle concentration of 4.5 mg/m^3. These data are summarized in Table 7-4.

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 (7.1, 3.5, or 0.35 mg DPM/m^3) for 7 hr/day, 5 days/week for up to 24 mo. Controls were exposed to filtered air. Diesel exhaust was provided by 5.7-L Oldsmobile V-8 engines operated continuously on the U.S. Federal Test Procedure urban certification cycle. The chambers were maintained at 25-28 °C, relative humidity at 40%-60%, and a flow rate sufficient for 15 air exchanges per hour. Animals were exposed during the light cycle, which ran from 6:00 AM to 6:00 PM. 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 for the low-exposure group. Contrary to what was observed in rats (Heinrich et al., 1986b; 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.

While earlier studies provided some evidence for tumorigenic responses in diesel-exposed mice, no increases were reported in the two most recent studies by Mauderly et al. (1996) and Heinrich et al. (1995), which utilized large group sizes and were well designed and conducted. Overall, the results in mice must therefore be considered to be equivocal.

7.3.1.3. *Hamster Studies*

Heinrich et al. (1982) examined the effects of diesel exhaust exposure on tumor frequency in female Syrian golden hamsters. Groups of 48 to 72 animals were exposed to clean air or whole diesel exhaust at a mean DPM concentration of 3.9 mg/m^3. Inhalation exposures were conducted 7 to 8 hr/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated under a constant load and a constant speed of 2,400 rpm. Flow rate was sufficient for about 20 exchanges per hour in the 250-L chambers. No lung tumors were reported in either exposure group.

In a subsequent study, Syrian hamsters were exposed 19 hr/day, 5 days/week for a lifetime to diesel exhaust diluted to a DPM concentration of 4.24 mg/m^3 (Heinrich et al., 1986b; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air or exhaust. No lung tumors were seen in either the clean-air group or in the diesel exhaust-exposed group.

In a third study (Heinrich et al., 1989b), 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. Exposures were conducted in chambers maintained at 22 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 animals were exposed 19 hr/day, 5 days/week beginning at noon each day, under a 12-hr light cycle starting at 7 AM. Forty animals per group were exposed to whole diesel exhaust or clean air. No lung tumors were detected in either the clean-air or diesel-exposed hamsters.

Brightwell et al. (1986, 1989) studied the effects of diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females, 6 to 8 weeks old, were exposed to diesel exhaust at DPM concentrations of 0.7, 2.2, or 6.6 mg/m^3. They were exposed 16 hr/day, 5 days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Section 7.3.1.1 and in Appendix A. No statistically significant (*t* test) relationship between tumor incidence and exhaust exposure was reported.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex in several studies of chronic duration at high exposure concentrations.

7.3.1.4. *Monkey Studies*

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m^3) for 7 hr/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. (1989) study with rats (Section 7.3.1.1) and are listed in 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.

7.3.2. Inhalation Studies (Filtered Diesel Exhaust)

Several studies have been conducted in which animals were exposed to diesel exhaust filtered to remove PM. Since these studies also included groups exposed to whole exhaust, details can be found in Sections 7.3.1.1 for rats, 7.3.1.2 for mice, and 7.3.1.3 for hamsters, and in Appendix A. Heinrich et al. (1986b) and Stöber (1986) reported negative results for lung tumor induction in female Wistar rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.24 mg/m^3. Negative results were also reported in female Fischer 344 rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.9 mg/m^3 (Iwai et al., 1986), in Fischer 344 rats of either sex exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 6.6 mg/m^3 (Brightwell et al., 1989), in female Wistar rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 7.0 mg/m^3 (Heinrich et al., 1995), and in female Fischer 344 rats exposed to filtered exhaust diluted to produce unfiltered particle concentrations of 5.1, 3.2, or 9.4 mg/m^3 (Iwai et al., 1997). In the Iwai et al. (1986) study, splenic lymphomas were detected in 37.5% of the exposed rats compared with 8.2% in controls.

In the study reported by Heinrich at al. (1986a) and Stober (1986), primary lung tumors were seen in 29/93 NMRI mice (males and females combined) exposed to filtered exhaust, compared with 11/84 in clean-air controls, a statistically significant increase. In a repeat study by Heinrich et al. (1995), however, significant lung tumor increases were not detected in either female NMRI or C57BL/6N mice exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.5 mg/m^3.

Filtered exhaust also failed to induce lung tumor induction in Syrian Golden hamsters (Heinrich et al., 1986a; Brightwell et al., 1989).

Although lung tumor increases were reported in one study and lymphomas in another, these results could not be confirmed in subsequent investigations. It is therefore concluded that little direct evidence exists for carcinogenicity of the vapor phase of diesel exhaust in laboratory animals at concentrations tested.

7.3.3. Inhalation Studies (Diesel Exhaust Plus Co-Carcinogens)

Details of the studies reported here have been described earlier and in Table 7-4. Tumor initiation with urethan (1 mg/kg body weight i.p. at the start of exposure) or promotion with butylated hydroxytolulene (300 mg/kg body weight i.p. week 1, 83 mg/kg week 2, and 150 mg/kg for weeks 3-52) did not influence tumorigenic responses in Sencar mice of both sexes exposed to concentrations of diesel exhaust up to 12 mg/m^3 (Pepelko and Peirano, 1983).

Heinrich et al. (1986b) exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 4 mg/m^3. See Section 7.3.1.1 for details of the exposure conditions. At the start of exposure the hamsters received either one dose of 4.5 mg diethylnitrosamine (DEN) subcutaneously per kg body weight or 20 weekly intratracheal instillations of 250 μg BaP. Female NMRI mice received weekly intratracheal instillations of 50 or 100 μgBaP for 10 or 20 weeks, respectively, or 50 μg dibenz[ah]anthracene (DBA) for 10 weeks. Additional groups of 96 newborn mice received one s.c. injection of 5 or 10 μg DBA between 24 and 48 hr after birth. Female Wistar rats received weekly subcutaneous injections of dipentylnitrosamine (DPN) at doses of 500 and 250 mg/kg body weight, respectively, during the first 25 weeks of exhaust inhalation exposure. Neither DEN, DBA, or DPN treatment enhanced any tumorigenic responses to diesel exhaust. Although response to BaP did not differ from that of BaP alone in hamsters, results were inconsistent in mice. Although 20 BaP instillations induced a 71% tumor incidence in mice, concomitant diesel exposure resulted in only a 41% incidence. However, neither 10 BaP instillations nor DBA instillations induced significant effects.

Takemoto et al. (1986) exposed Fischer 344 rats for 2 years to diesel exhaust at particle concentrations of 2 to 4 mg/m^3. One month after start of inhalation exposure one group of rats received di-isopropyl-nitrosamine (DIPN) administered i.p. at 1 mg/kg weekly for 3 weeks. Among injected animals autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were reported 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 adenocarcinomas was not significantly increased by exposure to diesel exhaust.

Brightwell et al. (1989) investigated the concomitant effects of diesel exhaust and DEN in Syrian hamsters exposed to diesel exhaust diluted to produce particle concentrations of 0.7, 2.2,

or 6.6 mg/m^3 for 2 years. The animals received a single dose of 4.5 mg DEN s.c. 3 days prior to start of inhalation exposure. DEN did not affect the lack of responsiveness to diesel exhaust alone. Heinrich et al. (1989b) also exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 3.75 mg/m^3 for up to 18 mo. After 2 weeks of exposure, groups were treated with either 3 or 6 mg DEN/kg body weight, respectively. Again, DEN did not significantly influence the lack of tumorigenic responses to diesel exhaust.

Heinrich et al. (1989a) investigated the effects of DPN in female Wistar rats exposed to diesel exhaust diluted to achieve a particle concentration of 4.24 mg/m^3 for 2-2.5 years. DPN at doses of 250 and 500 mg/kg body weight was injected subcutaneously once a week for the first 25 weeks of exposure. The tumorigenic responses to DPN were not affected by exposure to diesel exhaust. For details of exposure conditions of the hamster studies see Section 7.3.1.3.

Heinrich et al. (1986a) 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 hr/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.

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, particle-associated effects appear to be the primary cause of diesel-exhaust-induced lung cancer in rats exposed at high concentrations. This issue is discussed further in Chapter 7.

7.3.4. Lung Implantation or Intratracheal Instillation Studies

7.3.4.1. *Rat Studies*

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that PAHs in diesel exhaust that consist of four or more rings have

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 7-5). Fraction IIb (made up of PAHs with four to seven rings), which accounted for only 0.8% of the total weight of DPM condensate, produced the highest incidence of carcinomas following implantation into 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 activated carbon using the same dosing regime. 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.

Table 7-5. Tumor incidence and survival time of rats treated by surgical lung implantation 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.

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 was significantly greater in the DPM-instilled group and was significantly greater than the increase in the carbon-instilled group.

A study reported by Rittinghausen et al. (1997) suggested that organic constituents of diesel particles play a role in the induction of lung tumors in rats. An incidence of 16.7% pulmonary cystic keratinizing squamous cell lesions was noted in rats intratracheally instilled with 15 mg whole diesel exhaust particles, compared with 2.1% in rats instilled with 15 mg particles extracted to remove all organic constituents, and none among controls. Instillation of 30 mg of extracted particles induced a 14.6% incidence of squamous lesions, indicating the greater effectiveness of particles alone as lung particle overload increased.

Iwai et al. (1997) instilled 2, 4, 8, and 10 mg of whole diesel particles over a 2 to 10 week period into female F/344 rats, 50 or more per group. Tumors were reported in 6%, 20%, 43%, and 74% of the rats, with incidence of malignant tumors equal to 2%, 13%, 34%, and 48%, respectively. In a second experiment comparing whole with extracted diesel particles, tumor incidence equaled 1/48 (2%) in uninjected controls, 3/55 (5%) in solvent controls, 12/56 (21%) in extracted diesel particles, and 13/106 (12%) in animals injected with unextracted particles. Although the extracted particles appeared to be more potent, when converted to a lung burden basis (mg/100 mg dry lung) the incidence was only 14% among those exposed to extracted exhaust compared with 31% in those exposed to whole particles.

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 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, specific surface area increased to 138 m^2/gm. The MMAD for extracted PR was equal to 14 nm, while the specific surface area equaled 271 m^2/gm. The MMAD for extracted lampblack was equal to 95 nm, with a specific 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 lampblack 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 7-6.

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, toluene 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 particle surfaces had little detectable effect, suggesting that other organic components of diesel exhaust may be of greater importance in the induction of lung tumors at low doses pf B*a*P (0.2-0.4 mg).

7.3.4.2. *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 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

Table 7-6. 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.

Source: Dasenbrock et al., 1996.

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.

7.3.4.3. *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 the increase in tumor incidence in the 0.05 mg dose group, although less so with the other two. 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.

7.3.5. Subcutaneous and Intraperitoneal Injection Studies

7.3.5.1. *Mouse Studies*

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of i.p. injections of DPM on male (A/S) strain 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 eight-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—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 hr 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 hr 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.

7.3.6. Dermal Studies

7.3.6.1. *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 warmup 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 7-7. In the initial

Table 7-7. 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 warmup	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).

experiments using 52 (12 male, 40 female) C57BL mice treated with DPM extract from an engine operated in warmup mode, two papillomas were detected after 13 mo. Four tumors were detected 16 mo after the start of treatment in 8 surviving of 50 exposed male strain A mice treated with DPM extract from an engine operated under full load. 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 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.

Although these findings were not consistent with those of Kotin et al. (1955), the occurrence of a single carcinoma in a strain known to have an extremely low spontaneous tumor incidence may be of importance. Furthermore, a comparison between studies employing different strains of mice with varying spontaneous tumor incidences may result in erroneous assumptions.

Nesnow et al. (1982) studied the formation of dermal papillomas and carcinomas following dermal application of dichloromethane extracts from coke oven emissions, roofing tar, 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 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% 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 7-8. 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

Table 7-8. 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).

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.

7.3.7. 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 long-term inhalation bioassays with diesel exhaust have been carried out in the United States, Germany, Switzerland, and Japan, testing responses of rats, mice, and Syrian hamsters, and to a limited extent cats and monkeys.

It can be reasonably concluded that with adequate exposure, inhalation of diesel exhaust is capable of inducing lung cancer in rats. Responses best fit cumulative exposure (concentration × daily exposure duration × days of exposure). Examination of rat data shown in Table 7-9 indicates a trend of increasing tumor incidence at exposures exceeding 1×10^4 mg·hr/m^3. Exposures greater than approximately this value result in lung particle overload, characterized by slowed particle clearance and lung pathology, as discussed in Chapters 3 and 5, respectively. Tumor induction at high doses may therefore be primarily the result of lung particle overload with associated inflammatory responses. Although tumorigenic responses could not be detected under non-particle-overload conditions, the animal experiments lack sensitivity to determine if a threshold exists. If low-dose effects do occur, it can be hypothesized that the organic constituents are playing a role. See Chapter 7 for a discussion of this issue.

While rats develop adenomas, adenocarcinomas, and adenosquamous cell carcinomas, they also develop squamous keratinizing lesions. This latter spectrum appears for the most part to be peculiar to the rat. In a recent workshop aimed at classifying these tumors (Boorman et al., 1996), it was concluded that when these lesions occur in rats as part of a carcinogenicity study,

Table 7-9. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Mauderly et al.	35, 30	4,200	0	0	0	0.9
(1987)	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.	95, 35	13,300	0	0	0	0
(1986a)	95, 35	13,300	4.24	402.8	56,392	17.8
Heinrich et al.	90, 24	8,640	0	0	0	0
(1995)	90, 24	8,640	0.8	72.0	7,400	0
	90, 24	8,640	2.5	225.0	21,800	5.5
	90, 24	8,640	7.0	630.0	61,700	22.0
Ishinishi et al.	96, 30	11,520	0	0	0	3.3
(1988a)	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 7-9. Cumulative (concentration × time) exposure data for rats exposed to whole diesel exhaust (continued)

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$) Per week	Cumulative exposure ($mg \cdot hr/m^3$) Total	Tumor incidence (%)[a]
Brightwell et al.	80, 24	7,680	0	0	0	1.2
(1989)	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.	16, 18-24	1,152-1,536	0	0	0	0
(1986)	16, 18-24	1,152-1,536	2-4	32-64	3,456-4,608	0
Karagianes et al.	30, 20	2,400	0	0	0	0
(1981)	30, 20	2,400	8.3	249	19,920	16.6
Iwai et al. (1997)	56, 24	5,376	9.4	526	54,704	42
	48, 24	4,992	3.2	154	15,974	12
	54, 24	5,616	5.1	275	28,642	42

[a]Combined data for males and females.

they must be evaluated on a case-by-case basis and regarded as a part of the total biologic profile of the test article. If the only evidence of tumorigenicity is the presence of cystic keratinizing epitheliomas, it may not have relevance to human safety evaluation of a substance or particle. Their use in quantifying cancer potency is even more questionable.

The evidence for response of common strains of laboratory mice exposed under standard inhalation protocols is equivocal. Inhalation of diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986b; 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). However, in a repeat of their earlier study, Heinrich et al. (1995) failed to detect lung tumor induction in either NMRI or C57BL/6N mice. No increases in lung tumor rates were reported in a series of inhalation studies using strain A mice (Orthoefer et al., 1981; Kaplan et al., 1982; Kaplan et al., 1983; White et al., 1983). Finally, Mauderly et al. (1996) reported no tumorigenic responses in CD-1 mice exposed under conditions resulting in positive responses in rats. The successful induction of lung tumors in mice by Ichinose et al. (1997a,b) via intratracheal instillation may have been the result of focal deposition of larger doses. Positive effects in Sencar mice may be due to use of a strain sensitive to tumor induction in epidermal tissue by organic agents, as well as exposure from conception, although proof for such a hypothesis is lacking.

Attempts to induce significant increases in lung tumors in Syrian hamsters by inhalation of whole diesel exhaust were unsuccessful (Heinrich et al., 1982, 1986b, 1989b; Brightwell et al., 1986). However, hamsters are considered to be relatively insensitive to lung tumor induction. For example, while cigarette smoke, a known human carcinogen, was shown to induce laryngeal cancer in hamsters, the lungs were relatively unaffected (Dontenwill et al., 1973).

Neither cats (Pepelko and Peirano, 1983 [see Chapter 7]) nor monkeys (Lewis et al., 1986) developed tumors following 2-year exposure to diesel exhaust. The duration of these exposures, however, was likely to be inadequate for these two longer-lived species, and group sizes were quite small. Exposure levels were also below the maximum tolerated dose (MTD) in the monkey studies and, in fact, only borderline for detection of lung tumor increases in rats.

Long-term exposure to diesel exhaust filtered to remove particulate matter failed to induce lung tumors in rats (Heinrich et al., 1986b; Iwai et al., 1986; Brightwell et al., 1989), or in Syrian hamsters (Heinrich et al., 1986b; Brightwell, 1989). A significant increase in lung carcinomas was reported by Heinrich et al. (1986b) in NMRI mice exposed to filtered exhaust. However, in a more recent study the authors were unable to confirm earlier results in either NMRI or C57BL/6N mice (Heinrich et al., 1995). Although filtered exhaust appeared to potentiate the carcinogenic effects of DEN (Heinrich et al., 1982), nevertheless, because of the

lack of positive data in rats and equivocal or negative data in mice, it can be concluded that filtered exhaust is either not carcinogenic or has a low cancer potency.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of DPM. Rittinghausen et al. (1997) reported an increase in cystic keratinizing epitheliomas following intratracheal instillation of rats with either original DPM or DPM extracted to remove the organic fraction, with the unextracted particles inducing a slightly greater effect. 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. Intratracheal instillation did not induce lung tumors in Syrian hamsters (Kunitake et al., 1986; Ishinishi et al., 1988b).

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, which 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.

Results of experiments using tumor initiators such as DEN, B[a]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986b; Takemoto et al., 1986) were generally inconclusive 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 Section 3.6. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 4.

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. Additional studies by Heinrich et al. (1995) and Nikula et al. (1995) confirmed the capability of carbon particles to induce lung tumors. Induction of lung tumors by other particles of low solubility such as titanium dioxide (Lee et al., 1986) confirmed the capability of particles to induce 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., 1986a, 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 and intratracheal instillation data in rats and on i.p. 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. Exposure to filtered exhaust generally failed to induce lung tumors. 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 indicate a 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 primarily instrumental in the carcinogenic response observed in rats under sufficient exposure conditions. The ability of diesel exhaust to induce lung tumors at non-particle-overload conditions, and the relative contribution of the particles' core versus the particle-associated organics (if effects do occur at low doses) remains to be determined.

7.4. MODE OF ACTION OF DIESEL EMISSION-INDUCED CARCINOGENESIS

As noted in Chapter 2, diesel exhaust (DE) is a complex mixture that includes a vapor phase and a particle phase. The particle phase consists of an insoluble carbon core with a large

number of organic compounds, as well as inorganic compounds such as sulfates, adsorbed to the particle surface. Some of the semivolatile and particle-associated compounds, in particular PAHs, nitro-PAHs, oxy-PAHs, and oxy-nitro-PAHs (Scheepers and Bos, 1992), are considered likely to be carcinogenic in humans. The vapor phase also contains a large number of organic compounds, including several known or probable carcinogens such as benzene and 1,3-butadiene. Since exposure to the vapor phase alone, even at high concentrations, failed to induce lung cancer in laboratory animals (Heinrich et al., 1986), discussion will focus on the particulate matter phase. Additive or synergistic effects of vapor-phase components, however, cannot be totally discounted, since chronic inhalation bioassays involving exposure to diesel particles alone have not been carried out.

Several hypotheses regarding the primary mode of action of diesel exhaust have been proposed. Initially it was generally believed that cancer was induced by particle-associated organics acting via a genotoxic mechanism. By the late 1980s, however, studies indicated that carbon particles virtually devoid of organics could also induce lung cancer at sufficient inhaled concentrations (Heinrich, 1990). This finding provided support for a hypothesis originally proposed by Vostal (1986) that induction of lung tumors arising in rats exposed to high concentrations of diesel exhaust is related to overloading of normal lung clearance mechanisms, accumulation of particles, and cell damage followed by regenerative cell proliferation. The action of particles is therefore mediated by epigenetic mechanisms that can be characterized more by promotional than initiation stages of the carcinogenic process. More recently several studies have focused upon the production of reactive oxygen species generated from particle-associated organics, which may induce oxidative DNA damage at exposure concentrations lower than those required to produce lung particle overload. Since it is likely that more than one of these factors is involved in the carcinogenic process, a key consideration is their likely relative contribution at different exposure levels. The following discussion will therefore consider the possible relationship of the organic components of exhaust, inflammatory responses associated with lung particle overload, reactive oxygen species, and physical characteristics of diesel particles to cancer induction, followed by a hypothesized mode of action, taking into account the likely contribution of the factors discussed.

7.4.1. Potential Role of Organic Exhaust Components in Lung Cancer Induction

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). The majority of 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 such compounds identified from diesel exhaust are benzo(*a*)pyrene (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). More recently Enya et al. (1997) reported isolation of 3-nitrobenzanthrone, one of the most powerful direct-acting mutagens known to date, from the organic extracts of diesel exhaust.

Grimmer et al. (1987) separated diesel exhaust particle extract into a water- and a lipid-soluble fraction, and the latter was further separated into a PAH-free, a PAH-containing, and a polar fraction by column chromatography. These fractions were then tested in Osborne-Mendel rats by pulmonary implantation at doses corresponding to the composition of the original diesel exhaust. The water-soluble fraction did not induce tumors; the incidences induced by the lipid-soluble fractions were 0% with the PAH-free fraction, 25% with the PAH and nitro-PAH-containing fractions, and 0% with the polar fraction. The PAH and nitro-PAH-containing fraction, comprising only 1% by weight of the total extract, was thus shown to be responsible for most, if not all, of the carcinogenic activity.

Exposure of rats by inhalation to 2.6 mg/m^3 of an aerosol of tar-pitch condensate with no carbon core but containing 50 μg/m^3 benzo[*a*]pyrene along with other PAHs for 10 months induced lung tumors in 39% of the animals. The same amount of tar-pitch vapor condensed onto the surface of carbon black particles at 2 and 6 mg/m^3 resulted in tumor rates that were roughly two times higher (89% and 72%). Since exposure to 6 mg/m^3 carbon black almost devoid of extractable organic material induced a lung tumor rate of 18%, the combination of PAHs and particles increases their effectiveness (Heinrich et al., 1994). While this study shows the tumor-inducing capability of PAHs resulting from combustion, it should be noted that the benzo[*a*]pyrene content in the coal-tar pitch was about three orders of magnitude greater than in diesel soot. Moreover, because organics are present on diesel particles in a thinner layer and the particles are quite convoluted, they may be more tightly bound and less bioavailable. Nevertheless, these studies provide evidence supporting the involvement of organic constituents of diesel particles in the carcinogenic process.

Exposure of humans to related combustion emissions provides some evidence for the involvement of organic components. Mumford et al. (1989) reported greatly increased human lung cancer mortality in Chinese communes burning so-called smoky coal, but not wood, in unvented open-pit fires used for heating and cooking. Although particle concentrations were similar, PAH levels were five to six times greater in the air of communes burning smoky coal.

Coke oven emissions, containing high concentrations of PAHs but lacking an insoluble carbon core, have also been shown to be carcinogenic in humans (Lloyd, 1971).

Adsorption of PAHs to a carrier particle such as hematite, CB, aluminum, or titanium dioxide enhances their carcinogenic potency (Farrell and Davis, 1974). As already noted, 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. As discussed in Chapter 3, free organics are likely to be rapidly absorbed into the bloodstream, which may explain why the vapor-phase component of exhaust is relatively ineffective in the induction of pathologic or carcinogenic effects.

Even though the organic constituents may be tightly bound to the particle surface, significant elution is still likely because particle clearance half-times are nearly 1 year in humans (Bohning et al., 1982). Furthermore, Gerde et al. (1991) presented a model demonstrating that large aggregates 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 are 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.

B[*a*]P, although only one of many PAHs present in diesel exhaust, is the one most extensively studied. Bond et al. (1983, 1984) demonstrated metabolism of particle-associated B[*a*]P and free B[*a*]P by alveolar macrophages (AM) and by type II alveolar cells. The respiratory tract cytochrome P-450 systems have an even greater concentration in the nonciliated bronchiolar cells (Boyd, 1984). It is worthy to note that bronchiolar adenomas that develop following diesel exposure have been found to resemble both Type II and nonciliated bronchiolar cells. It should also be noted that any metabolism of procarcinogens by these latter two cell types probably involves the preextraction of carcinogens in the extracellular lining fluid and/or other

endocytotic cells, since they are not especially important in phagocytosis of particles. Thus, bioavailability is an important issue in assessing the relative importance of PAHs.

Additionally, a report by Borm et al. (1997) indicates that incubating rat lung epithelial-derived cells with human PMNs (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to benzo[*a*] pyrene; addition of more activated PMN in relation to the number of lung cells further increased adduct formation in a dose-dependent manner. The authors suggest that "an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particles." These data raise the possibility that DE exposure at low concentrations may result in levels of neutrophil influx that would not necessarily be detectable via histopathological examination as acute inflammation, but which might be effective at amplifying any potential diesel exhaust genotoxic effect.

Nitro-PAHs have also been implicated as potentially involved in diesel-exhaust-induced lung cancer. Although the nitro-PAH fraction of diesel was less effective than PAHs in the induction of lung cancer when implanted into the lungs of rats (Grimmer et al., 1987), in a study of various extracts of diesel exhaust particles, 30%-40% of the total mutagenicity could be attributed to a group of six nitroarenes (Salmeen et al., 1984). Moreover, Gallagher et al. (1994) reported results suggesting that DNA adducts are formed from nitro-PAHs present in DNA and may play a role in the carcinogenic process. Nitroarenes, however, quantitatively represent a very small percentage of diesel particle extract (Grimmer et al., 1987), making their role in the tumorigenic response uncertain.

The induction of DNA adducts in humans occupationally exposed to diesel exhaust indicates the likelihood that PAHs are participating in the tumorigenic response, and that these effects can occur at exposure levels less than those required to induce lung particle overload. 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 significantly elevated levels of DNA adducts in lymphocytes from garage workers with known diesel exhaust exposure compared to unexposed mechanics. 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.

7.4.2. Role of Inflammatory Cytokines and Proteolytic Enzymes in the Induction of Lung Cancer by Diesel Exhaust

It is well recognized that the deposition of particles in the lung can result in the efflux of polymorphonuclear leucocytes (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). During chronic exposure the numbers of PMNs lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled diesel particulate matter (DPM) concentration (Strom, 1984). Strom (1984) also found that PMNs in diesel-exposed lungs remained persistently elevated for at least 4 months after cessation of exposure, a potential mechanism that may be related to an ongoing release of phagocytized particles. Evidence in support of this possibility was reported 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 became progressively elevated in the lungs of the animals in which alveolar-phase clearance was inhibited. Moreover, the particle burdens in the PMNs became progressively greater over time. Such findings are consistent with an ongoing particle relapse process, in which particles released by dying phagocytes are ingested by new ones.

The inflammatory response, characterized by efflux of PMNs from the vascular compartment, is mediated by inflammatory chemokines. Driscoll et al. (1996) reported that inhalation of high concentrations of carbon black stimulated the release of macrophage inflammatory protein 2 (MIP-2) and monocyte chemotactic protein 1 (MCP-1). They also reported a concomitant increase in hprt mutants. In a following study it was shown that particle exposure stimulates production of tumor necrosis factor TNF-α, an agent capable of activating expression of several proteins that promote both adhesion of leucocytes and chemotaxis (Driscoll et al., 1997). In addition, alveolar macrophages also 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; Driscoll et al., 1996).

Another characteristic of AMs and PMNs under particle overload conditions is the release of 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 common feature observed in 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 cycling of target cells and the probability of a neoplastic-associated genomic disturbance.

7.4.3. Role of Reactive Oxygen Species in Lung Cancer Induction by Diesel Exhaust

Phagocytes from a variety of species produce elevated levels of oxidant reactants in response to challenges, with the physiochemical characteristics of a phagocytized particle being a major factor in determining the magnitude of the oxidant-producing response. Active oxygen species released by the macrophages and lymphatic cells can cause lipid peroxidation in the membrane of lung epithelial cells. These lipid peroxidation products can initiate a cascade of oxygen free radicals that progress through the cell to the nucleus, where they damage DNA. If this damage occurs during the epithelial cell's period of DNA synthesis, there is some probability that the DNA will be replicated unrepaired (Lechner and Mauderly, 1994). The generation of reactive oxygen species by both AMs and PMNs should therefore 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.

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. Cellular DNA is damaged by oxygen free radicals generated from a variety of sources (Ames, 1983; Trotter, 1980). Along this line, Weitzman and Stossel (1981) found that human peripheral leukocytes are 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).

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). 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). Although these responses may occur at any concentration, they are likely to be greatly enhanced at high exposure concentrations with slowed clearance and lung particle overload.

Reactive oxygen species can also be generated from particle-associated organics. Sagai et al. (1993) reported that DPM can nonenzymatically generate active oxygen species (such as superoxide [O_2^-] and hydroxyl radical [·OH] in vitro without any biologically activating systems) such as microsomes, macrophages, hydrogen peroxide, or cysteine. Because DPM washed with methanol could no longer produce these radicals, it was concluded that the active components were compounds extractable with organic solvents. However, the nonenzymatic contribution to the DPM-promoted active oxygen production was negligible compared to that generated via an enzymatic route (Ichinose et al., 1997a). They reported that O_2^- and ·OH can be enzymatically generated from DPM by the following process. Soot-associated quinone-like compounds are reduced to the semiquinone radical by cytochrome P-450 reductase. These semiquinone radicals then reduce O_2 to O_2^-, and the produced superoxide reduces ferric ions to ferrous ions, which catalyzes the homobiotic cleavage of H_2O_2 dismutated from O_2 by superoxide dismutase or spontaneous reactions to produce ·OH. According to Kumagai et al. (1997), while quinones are

likely to be the favored substrates for this reaction, the participation of nitroaromatics cannot be ruled out.

One of the critical lesions to DNA bases generated by oxygen free radicals is 8-hydroxydeoxyguanosine (8-OHdG). The accumulation of 8-OhdG as a marker of oxidative DNA damage could be an important factor in enhancing the mutation rate leading to lung cancer (Ichinose et al., 1997a). For example, formation of 8-OHdG adducts leads to G:C to T:A transversions unless repaired prior to replication. Nagashima et al. (1995) demonstrated that the production of (8-OHdG) is induced in mouse lungs by intratracheal instillation of DPM. Ichinose et al. (1997b) reported further that while intratracheal instillation of DPM in mice induced a significant increase in lung tumor incidence, comparable increases were not reported when mice were instilled with extracted DPM (to remove organics). Lung injury was also less in the mice instilled with extracted DPM. Moreover, increases in 8-OHdG in the mice instilled with unextracted DPM correlated very well with increases in tumor rates. In a related study, Ichinose et al. (1997a) intratracheally instilled small doses of DPM, 0.05, 0.1, or 0.2 mg weekly for 3 weeks, in mice fed standard or high-fat diets, either with or without β-carotene. High dietary fat enhanced DPM-induced lung tumor incidence, while β-carotene, which may act as a free radical scavenger, partially reduced the tumorigenic response. Formation of 8-OHdG was again significantly correlated with lung tumor incidence in these studies, except at the highest dose. Dasenbrock et al. (1996) reported that extracted DPM, intratracheally instilled into rats (15 mg total dose) induced only marginal increases in lung tumor induction, while unextracted DPM was considerably more effective. While adducts were not measured in this study, it nevertheless provides support for the likelihood that either activation of organic metabolites and/or generation of oxygen free radicals from organics are involved in the carcinogenic process.

Additional support for the involvement of particle-associated radicals in tissue damage was provided by the finding that pretreatment with superoxide dismutase (SOD), an antioxidant, markedly reduced lung injury and death due to instillation of DPM. Similarly Hirafuji et al. (1995) found that the antioxidants catalase, deferoxamine, and MK-447 inhibited the toxic effects of DPM on guinea pig tracheal cells and tissues in vitro.

Although the data presented supported the hypothesis that generation of reactive oxygen species resulting from exposure to DPM is involved in the carcinogenic process, it should be noted that 8-OHdG is efficiently repaired and that definitive proof of a causal relationship in humans is still lacking. It is also uncertain whether superoxide or hydroxyl radicals chemically generated by DPM alone promote 8-OHdG production in vivo and induce lung toxicity, because SOD is extensively located in mammalian tissues. Nevertheless, demonstration that oxygen free radicals can be generated from particle-associated organics, that their presence will induce adduct

formation and DNA damage unless repaired, that tumor induction in experimental animals correlated with OhdG adducts, and that treatment with antioxidant limits lung damage, provides strong support for the involvement of oxygen free radicals in the toxicologic and carcinogenic response to diesel exhaust.

7.4.4. Relationship of Physical Characteristics of Particles to Cancer Induction

The biological potential of inhaled particles is strongly influenced by surface chemistry and character. For example, the presence of trace metal compounds such as aluminum and iron, as well as ionized or protonated sites, is important in this regard (Langer and Nolan, 1994). A major factor is specific surface area (surface area/mg). PMNs 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 spherical TiO_2 particles in the 1-2 μm diameter range (Strom, 1984; Lee et al., 1986). In these studies lung tumors were detected only at an inhaled concentration of 250 $\mu g/m^3$. In a more recent study in which rats were exposed to TiO_2 in the 15-40 nm size range, inhibition of particle clearance and tumorigenesis were induced at concentrations of 10 mg/m^3 (Heinrich et al., 1995). Oberdörster and Yu (1990) compared the results of several chronic inhalation studies and found that carcinogenic potency related to specific particle surface area. Heinrich et al. (1995) also found that lung tumor rates increased with specific particle surface area following exposure to diesel exhaust, carbon black, or titanium dioxide, irrespective of particle type. Langer and Nolan (1994) reported that the hemolytic potential of Min-U-Sil15, a silica flour, increased in direct relationship to specific surface area at nominal particle diameters ranging from 0.5 to 20 μm.

Ultrafine particles appear to be more likely to be taken up by lung epithelial cells. 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.

The carcinogenic potency of diesel particles, therefore, appears to be related, at least to some extent, to their small size and convoluted shape, which results in a large specific particle surface area. While toxicity and carcinogenicity increased with decreasing particle size into the submicron range, it is uncertain if toxic and carcinogenic potential continues to increase as particle size decreases even further. The relationship between particle size and toxicity is of concern because, as noted in Chapter 2, newer engines equipped with more advanced emission

controls emit greater numbers of particles in the nanometer size range. Other than disruption of the cytoskeleton of epithelial cells, there is little information regarding the means by which particle size influences carcinogenicity as well as noncancer toxicity.

7.4.5. Integrative Hypothesis For Diesel-induced Lung Cancer

The induction of lung cancer by large doses of carbon black via inhalation (Heinrich et al., 1995; Mauderly et al., 1991; Nikula et al., 1995) or intratracheal instillation (Kawabata et al., 1994; Pott et al., 1994; Dasenbrock et al., 1996) led to the development of the lung particle overload hypothesis. According to this hypothesis the induction of neoplasia by insoluble, biochemically inert particles is associated with an inhibition of lung particle clearance and the involvement of persistent alveolar epithelial hyperplasia. Driscoll (1995), Driscoll et al. (1996), and Oberdörster and Yu (1990) 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), 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. A schematic of this hypothesis has been outlined by McClellan (1997) (see Figure 7-5). In support of this hypothesis, it was reported that concentrations of inhaled CB resulted in increased cytokine expression and inflammatory influx of neutrophils (Oberdörster et al., 1995), increased formation of 8-OhdG (Ichinose et al., 1997b), and increase in the yield of hprt mutants, an effect ameliorated by treatment with antioxidants (Driscoll, 1995; Driscoll et al., 1996). Metabolism of carcinogenic

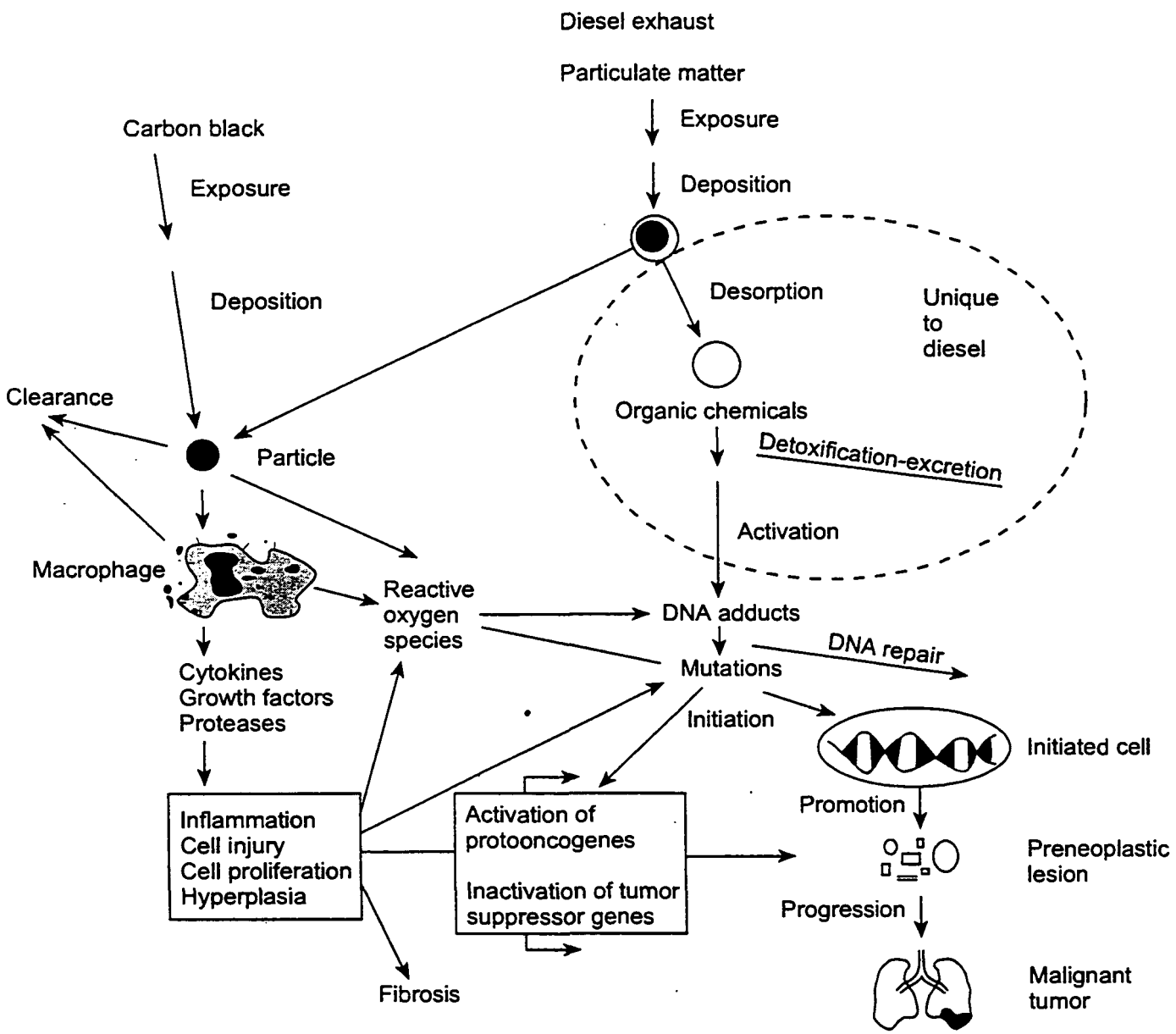


Figure 7-5. Pathogenesis of lung disease in rats with chronic, high-level exposures to particles.

Source: McClellan, 1997.

organics to active forms as well as the generation of reactive oxygen species from certain organic species are likely to contribute to the toxic and carcinogenic process.

At low concentrations, inflammatory effects associated with lung particle overload are generally absent. However, activation of organic carcinogens and generation of oxidants from the organic fraction can still be expected. Actual contribution depends upon elution and the effectiveness of antioxidants. Direct effects of ultrafine diesel particles taken up by epithelial cells are also likely to play a role.

While high-dose induction of cancer is logically explained by this hypothesis, particle overload has not been clearly shown to induce lung cancer in other species. As noted in the quantitative chapter, the relevance of the rat pulmonary response is therefore problematic. The rat pulmonary noncancer responses to DPM, however, have fairly clear interspecies and human parallels. In response to poorly soluble particles such as DPM, human and rats both develop an alveolar macrophage response, accumulate particles in the interstitium, and show mild interstitial fibrosis (ILSI, 1999). Other species (mice, hamsters) also have shown similar noncancer pulmonary responses to DPM, but without accompanying cancer response. The rat response for noncancer pulmonary histopathology, however, seems to be more pronounced compared to humans or other species, i.e., rats appear to be more sensitive. Although many critical elements of interspecies comparison such as the role of airway geometry and patterns of particle deposition need further elucidation, this basic interspecies similarity and greater sensitivity of pulmonary response make pulmonary histopathology in rats a valid basis for noncancer dose-response assessment.

7.4.6. Summary

Recent studies have shown tumor rates resulting from exposures to nearly organic-free CB particles at high concentrations to be similar to those observed for DE exposures, thus providing strong evidence for a particle overload mechanism for DE-induced pulmonary carcinogenesis in rats. Such a 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.

The particle overload hypothesis appears sufficient to account for DE-induced lung cancer in rats. However, there is increasing evidence for lung cancer induction in humans at concentrations insufficient to induce lung particle overload (see Chapter 7). Uptake of particles by epithelial cells at ambient or occupational exposure levels, DNA damage resulting from oxygen-free radicals generated from organic molecules, and the gradual in situ extraction and activation of procarcinogens associated with the diesel particles are likely to play a role in this

response. The slower particle clearance rates in humans (up to a year or more) increase the likelihood of significant extraction of organics. This is supported by reports of increased DNA adducts in humans occupationally exposed to diesel exhaust at concentrations unlikely to induce lung particle overload. While these modes of action can be expected to function at lung overload conditions also, they are likely to be overwhelmed by inflammatory associated effects.

The evidence to date indicates that 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. The likelihood that different modes of action predominate at high and low doses also renders low-dose extrapolation to ambient concentrations uncertain.

7.5. CANCER WEIGHT-OF-EVIDENCE: HAZARD EVALUATION

7.5.1. Cancer Hazard Summary

Diesel engine exhaust is *"highly likely"* to be carcinogenic by the inhalation route of exposure, according to EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment. The hazard is viewed as being applicable to ambient-environmental exposures. There is no available evidence to evaluate the hazard from other routes of exposure. The "likely" classification generally compares with other agents designated as "B-1 probable human carcinogen" under the EPA's 1986 Guidelines for Carcinogen Risk Assessment, though the overall weight-of-evidence for diesel exhaust (DE) places it at the upper end of this grouping and hence gives it a "highly likely" designation under the proposed Guidelines. The carcinogenic potential of DE is indicated by (1) a consistent statistically increased association between observed lung cancer and DE exposure in certain occupationally exposed workers, (2) the induction of lung cancer in some but not all animal experiments, (3) mutagenic and carcinogenic activity of the particle organic extracts, (4) the presence of individual organic compounds having known mutagenic and/or carcinogenic properties (e.g., PAH derivatives and nitro-PAHs), and (5) limited evidence for the bioavailability of the organics. The mode of action for carcinogenicity in humans is unknown; it is suspected that either the organics, the elemental carbon diesel particle, or both contribute to the carcinogenic activity.

Increases in relative risk for lung cancer have been consistently noted in a number of epidemiologic studies, and causality considerations for this observed association are consistent with DE exposure being causally related to lung cancer. An inability to satisfactorily minimize all confounding, bias, and exposure uncertainties, coupled with the magnitude of the relative

risks, limits the human evidence from being considered sufficient to characterize DE as a "known" human carcinogen.

While lung cancer has been induced experimentally in rats via inhalation of DE at high exposure concentrations, and in rats and mice via intratracheal instillation of diesel particles and particle extracts, these responses appear to be mediated primarily by inflammation and subsequent pathology related to lung particle overload. Because the persistent-chronic overload inflammatory responses in the rat are not seen at lower test exposures (or at ambient DE concentrations), and uncertainty remains whether induction of inflammatory responses in humans will lead to lung cancer, rat bioassay data are not completely irrelevant for human hazard characterization. However, the rat lung cancer response data are unsuitable for estimating human risk at environmental levels of exposure.

The plausibility of an environmental hazard is supported by (1) considering that mutagenic compounds and tumor-initiating carcinogens (e.g., PAH derivatives and nitro-PAHs) are present in small quantities in the DE organic mixture, which qualitatively implies a nonthreshold mode of action for these agents; and (2) noting that there could be little difference between higher-end environmental exposures and some occupational levels where increased relative risks of about 1.4 are seen (e.g., exposure estimates for some truck drivers could be overlapping some environmental estimates, they also may have somewhat higher relative risks). For these reasons, the extrapolation of the occupational hazard to ambient environmental exposures is judged plausible and prudent. In the absence of evidence to the contrary, and recognizing the mutagenic potential of the organics, it would also be feasible to evaluate dose response using linear models, at least at low exposure levels.

Overall, the evidence for a likely human carcinogenic hazard by inhalation is strong, even though inferences are involved. Uncertainties remain, including (1) methodologic limitations in the epidemiologic studies as well as a lack of assured historical exposure data for occupationally exposed cohorts, (2) uncertainties regarding the extent of bioavailability of organic compounds present on diesel particles, and (3) uncertainties regarding the mode of action in humans.

7.5.2. Supporting Information

7.5.2.1. *Human Data*

An increased relative risk for lung cancer and DE exposure has been observed in more than 30 epidemiologic studies. The excess risk is observed in both cohort and case-control study designs. Additionally, consistent and statistically significant elevated pooled relative risks ranging from 1.33 to 1.47 were derived in several meta-analyses. In some studies, the effects of

smoking were accounted for and the increased relative risks prevailed. When the meta- analysis focused only on the smoking-controlled studies, the relative risks tended to increase. A few individual studies had smoking-adjusted relative risks exceeding 1.5 (e.g., Steenland et al., 1990 [RR 1.64, 1.89]). The uncertainties with the epidemiologic data are typical ones including the possibility that chance, bias, or confounding are influencing the observed lung cancer increases. The persistence of this association in so many studies indicates that chance alone is unlikely to account for the observed relation between DE and lung cancer. A causal interpretation for DE is enhanced when the "Hill" causality criteria are evaluated, noting that a weakness or absence in one or several of the criteria does not prevent a causal interpretation. A weakness in the epidemiologic studies is due to the fact that the information from which diesel exposure can be inferred is based on job codes, area descriptions, etc., which are surrogates for the true underlying exposure. This can lead to "nondifferential" misclassification of exposure, and while unlikely, might result in a spurious risk estimate in any one study. It is even more unlikely, however, that it would bias a sufficient number of studies in a uniform direction to account for the persistent aggregate association observed. Moreover, any bias would likely be toward a lower risk estimate. In those studies where the confounding effect of smoking was controlled, there remains the suspicion that the statistical adjustment for smoking may not be completely effective, and residual confounding by smoking may persist to bias the correlation of DE exposure with lung cancer occurrence.

7.5.2.2. *Animal Data*

Numerous studies have shown that inhalation of DE and intratracheal instillation of diesel particles or particle extract result in the induction of lung cancer in rats. Although evidence of lung cancer induction from DE in mice via inhalation exposure is equivocal, positive results have been obtained by intratracheal instillation of diesel particles. Attempts to induce lung cancer in Syrian hamsters by inhalation of DE have been unsuccessful, but this species is known to be resistant to the induction of lung cancer. Although cats and monkeys have been exposed to DE, the durations of exposure were inadequate to evaluate carcinogenicity. As supported by an expert panel (ILSI, 1998), the high-dose rat data are unsuitable for predicting a low-exposure human risk. Because it is unknown whether high lung burdens of poorly soluble particles (e.g., diesel particles) can lead to lung cancer in humans via mechanisms similar to those of the rat, there are insufficient data to conclude that the rat response is completely irrelevant for human hazard identification. Intratracheal instillation studies in rats and mice reveal that diesel particles as well as the particle organic extracts can elicit a lung cancer response.

7.5.2.3. *Other Key Data*

Organic extracts of DE particles have been shown to induce tumors in mice, both by skin painting and subcutaneous injection, and to be mutagenic in several test systems. Additionally, a number of PAHs and nitro-PAHs present on diesel particles as well as in the vapor phase are known to be mutagenic and/or carcinogenic. Further evidence for the presence of carcinogenic agents in DE is provided by the reported induction of dermal tumors following skin painting of diesel particle extracts.

7.5.2.4. *Mode of Action*

The mode of action for DE carcinogenicity, especially at nonparticle overload exposure conditions, remains to be established. There is some evidence and thus plausibility that diesel particles as well as particle-associated organics are involved in the carcinogenic process. The rat model shows that at high-exposure concentrations, particle-overload-induced inflammatory responses, associated DNA damage, and rapid cell turnover are likely the primary factors responsible for lung cancer induction. It is not known whether humans have a similar response pattern at high exposures, though it has not been historically observed. At low exposure levels, cancer induction is more likely to be due to organic compounds, although there is some evidence that ultrafine diesel particles at low concentrations are ingested by epithelial cells and induce DNA damage. DNA damage in blood cells of occupationally exposed workers indicates at least some degree of elution of organic compounds from the particle and subsequent entry into the bloodstream. Studies have also suggested that bioavailability may be greater at low-exposure concentrations because the particles are not aggregated. A significant percentage of particles in humans are deposited at the branchings of small airways rather than alveoli, and the residence time of organic compounds eluted at those locations is greater, increasing the likelihood of metabolism to an activated state. A variety of carcinogenic compounds (e.g., PAH derivatives and nitro-PAHs), a number of which are mutagens and carcinogens, are present on the diesel particle. It has also been shown that reactive oxygen species capable of damaging DNA are generated by the metabolism of DE organics with quinone-like structures.

7.6. DISCUSSION OF THE ROLE OF DIESEL EXHAUST IN THE OVERALL PICTURE OF PM_{10}

It is very difficult to assess exposure to diesel emissions because they are highly complex mixtures and constitute only a small portion of a broader mix of air pollutants. For example, combustion of other materials, such as fossil fuel and tobacco, produces many of the same chemical components present in diesel emissions; furthermore, both natural and manmade

sources of respirable particles are common. No single constituent of diesel exhaust serves as a unique marker of exposure.

Whether air pollution contributes to the occurrence of lung cancer is a matter of wide debate. Ambient air in urban or industrialized areas can be contaminated by chemicals, some of which are definitely known to be carcinogenic. Known carcinogens that occur in ambient air include arsenic, asbestos, benzene, cadmium, and polycyclic aromatic hydrocarbons. However, the information on ambient exposure is sparse. Detailed measurements of such substances over long exposure periods and for large geographic areas are rarely available. Many descriptive epidemiologic studies demonstrate increased lung cancer risk in urban and industrialized areas (Hemminki, 1994). Frequently, those differences have been partially explained by differences in air pollution; however, such correlations might have other explanations and do not represent final conclusive evidence. The coincidence of diesel exhaust exposure and air pollution in urban airsheds poses important questions about whether the observed association of an increased lung cancer risk in urban and industrialized areas can be attributed to diesel exhaust exposure.

The contribution of diesel particles to PM_{10} (particles ≤ 10 *um* diameter) are difficult to determine, although most estimates indicate they constitute only a small fraction. For example, in an analysis conducted in the Los Angeles basin in the early 1980s, diesel emissions accounted for approximately 3% of the mass of PM_{10}. Because 90% of diesel particles are less than 1 *um* diameter they make up a larger percentage of fine and ultrafine ambient PM. For example, the EPA has estimated that diesel particles accounts for 5.7% of all $PM_{2.5}$ and 21% of $PM_{2.5}$ excluding natural and fugitive dust sources (see Chapter 2 for details). Since smaller particles appear to be more toxic/carcinogenic, the size distribution as well as mass of diesel particles relative to other PM are important considerations in any attempt to estimate the contribution of DE to PM induced toxicity and/or carcinogenicity.

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8. CANCER DOSE-RESPONSE EVALUATION

8.1. INTRODUCTION

This chapter discusses the dose-response aspects of the key carcinogenicity data. The identification of a dose-response can enhance the understanding of the cancer hazard and lead to an estimation of possible disease impact. One measure of impact used by EPA is the cancer unit risk. Unit risk is the estimated cancer risk at 1 $\mu g/m^3$ of exposure, in this case $\mu g/m^3$ of diesel exhaust (DE) particulate matter, from a continuous 70-year exposure. Unit risk derivation procedures and specifications are defined in EPA's risk assessment guidance (U.S. EPA, 1986, 1996).

Evidence shows that DE is likely to pose a human hazard for lung cancer by the inhalation route of exposure. The mode of action (MoA) for humans has not been determined, and the presumed MoA for rats does not justify using these data to estimate cancer unit risk for humans. EPA believes that a role for both mutagenic effects and particle-specific effects is plausible. According to EPA Cancer Guidelines, the mutagenic effects would allow the modeling of dose-response using models with a linear term at low doses. With toxicologically suspect organics thought to be in proportion to the mass of particulates, the use of $\mu g/m^3$ of DE particles as the dosimeter is feasible. With no clear indication that key organic components have changed disproportionately to the particle mass over the years (note that the overall particle mass has been decreasing), the use of older toxicological results based on older engine exposures to predict current-day hazards is also feasible, though uncertainty exists in this assumption. The overall challenge with DE is to judge the uncertainties going into the dose-response analysis and decide whether to proceed and, if so, what certainties/uncertainties to ascribe to any resulting analysis and follow-on unit risk derivation. For DE, the cascade of desirable to less desirable approaches clearly starts with human data, then to comparative potency approaches that use various surrogate exposure-response relationships.

For a variety of reasons EPA is not, at this time, adopting or recommending a cancer unit risk or risk range for DE. EPA will monitor ongoing research and reanalysis of epidemiology-exposure studies and may revisit dose-response and unit risk derivation.

8.2. REVIEW OF PREVIOUS QUANTITATIVE RISK ESTIMATES

Early attempts to quantitatively assess the carcinogenicity of diesel engine emissions were hindered by a lack of positive epidemiologic studies and long-term animal studies. One means of overcoming these obstacles was the use of the so-called comparative potency method (Albert et al., 1983). An attempt to estimate risk based on human data was also made at this time by Harris (1983), although it was based upon equivocal evidence. By the late 1980s the availability of data

from animal bioassays and epidemiologic studies provided an opportunity to the derivation of both animal and human data-based estimates. See Table 8-1 for a historical overview.

8.2.1. Comparative Potency Method

In the comparative potency method, a combustion or pyrolysis product is selected that has 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 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

Table 8-1. 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
Multistage model	8.9×10^{-5}	Lung cancer rats[f]	CAL-EPA, 1998
Multistage model	3.4×10^{-5}	Lung cancer rats[d]	WHO, 1996
Biological model	1.7×10^{-5}	Lung cancer rats[d]	Chen and Oberdorster, 1996
Biological model	3.5×10^{-6}	Assuming particle threshold	Chen and Oberdorster, 1996
Epidemiologic analysis	1.4×10^{-3}	London transport study	Harris, 1983
Epidemiologic analysis	6×10^{-4}	Railroad workers	CAL-EPA, 1998
Epidemiologic analysis	$0.6\text{-}2 \times 10^{-3}$	Railroad workers	U.S. EPA, 1998
Epidemiologic analysis	1.6×10^{-2}	Truck drivers[g]	Steenland et al., 1998

[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.
[f]Used data from studies by Brightwell, et al., 1989; Heinrich et al., 1995; Ishinishi et al., 1986; Mauderly, 1987; and Nikula et al., 1995.
[g]Estimated risk of 45 years occupational exposure to 5 μg/m³.

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 with 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.

8.2.2. Suitability of Comparative Potency Approach

As noted earlier, in this method the potency of DPM extract is compared with other combustion or pyrolysis products, for which epidemiology-based unit risk estimates have been developed. Comparisons are made using short-term tests such as skin painting, mutations, 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.

Although this test was based originally on the belief that cancer induction at low doses is due to the organic fraction present on the diesel particles, it is possible to argue, through a biologically based dose-response modeling concept, that the relative cancer risk of two compounds is approximately equal to the ratio of initiation rate of the two compounds at low doses even though particles may assert other effects at higher doses; thus, making it a reasonable approach for risk derivation. A major strength of this approach is avoidance of lung particle overload effects. Furthermore, independent tests have shown that the organic fraction of DE may damage DNA and thus may induce cancer (see Chapter 7). Finally, the carcinogenic potency of the organic fraction can be compared with related emissions for which cancer potency is reasonably well defined.

A major uncertainty of this approach is the assumption that cancer potency can be determined on the basis of the effectiveness of the organic fraction alone. Under lung particle overload conditions, particles are considered to play a primary role in lung cancer induction. As

noted in Chapter 4, ultrafine diesel particles may be ingested by epithelial cells even at low concentrations, inducing damage to the genetic material and possible carcinogenic effects. The potency estimates using this approach may therefore underestimate risk by not accounting for possible effects of particles or even reactive oxygen species. Association of organics with particles may also influence their potency depending on relative elution rates, efficiency of activation, etc. A final uncertainty involves the assumption that potency in short-term tests is an accurate predictor of lung cancer potency.

The uncertainties of this approach preclude its unilateral adoption for predicting upper-bound estimates.

8.2.3. Animal Bioassay-Based Cancer Potency Estimates

With the availability of chronic cancer bioassays, a considerable number of potency estimates were derived using 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 for 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 (LMS) 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. The EPA (1998) derived a unit risk estimate of $3.4 \times 10^{-5}/\mu g/m^3$, based on lung burden of DPM per unit lung surface area, using an LMS model and calculating the geometric mean from results of bioassay data reported by Mauderly et al. (1987), Ishinishi et al. (1986), and Brightwell et al. (1989). California EPA (OEHHA, 1998) derived a geometric mean estimate of $6 \times 10^{-5}/\mu g/m^3$ from five bioassays using an LMS model.

In an attempt to demonstrate the possible influence of particle effects as well as particle-associated organics, an additional modeling approach was attempted by Chen and Oberdorster

(1996). Employing a biologically based two-stage model and using malignant tumor data from Mauderly et al. (1997), the upper bound risk estimate for exposure to 1 $\mu g/m^3$ was estimated to be 1.7×10^{-5}. This estimate is virtually identical to that using the LMS model, assuming nonthreshold effect of particles. If a threshold of particle effect is assumed, however, the estimated risk decreases about fivefold. The results also show that the mechanism of diesel-induced lung tumor at high exposure concentrations may differ from that at low exposure concentrations, with organics and particles playing primary roles of tumorigenesis respectively at low and high concentrations.

8.2.4. Suitability of Laboratory Animal Bioassay Approach

Cancer risk assessment from exposure to DE, based on available animal bioassays, traditionally has strengths and uncertainties. For DE the best studies are adequately designed, eliminating confounding factors often present in epidemiology studies. Exposure duration and exposure levels can be precisely controlled and monitored. The presence or absence of tumors can be verified by pathological evaluation. Although animal-to-human extrapolation of dose is required and has uncertainty, 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 a consensus of experts that the rat seems to be unique in its response to particulate matter, and therefore its use for assessing human lung cancer risk is problematic (ILSI, 1998; Mauderly, 1994). As noted in Chapter 7, the rat is the only species that has unequivocally been shown to develop lung cancer in response to inhaled DE. However, what is happening in the human lung is uncertain. It has also been argued that humans are more 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), though this study 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). 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.

Although rat data may still have limited value for hazard identification they are much less suitable for quantitating human environmental risk. For example, 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 larger extent at the bifurcation of the small bronchi. Differing deposition patterns are likely to result in different pathologic responses, as reported by Nikula et al. (1997) for rats and monkeys.

Another major uncertainty in the use of rat bioassay data concerns extrapolation of lung cancer to ambient concentrations. Significant lung tumor increases in experimental animals have generally been obtained only at concentrations resulting in lung particle overload with concomitant pathological effects. As discussed in Section 7.4, it has been hypothesized that lung cancer induction results from a secondary effect associated with 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 (Driscoll, 1995).

If the primary means of lung cancer induction in rats is via particle-overload mechanisms, then it can be surmised that different factors are plausibly responsible for induction of lung cancer in humans exposed at occupational or ambient concentrations. Experimental evidence provides some support for the existence of 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. Ichinose (1997a, b) reported that not only are reactive oxygen species generated from organics present on the surface of diesel particles, but the production of these radicals is well correlated with increased in 8-hydroxydeoxyguanine adducts. Finally, Dasenbrock et al. (1996) reported that extraction of the organic fraction from diesel particles decreased their carcinogenic potency, suggesting a role for organic constituents. Because the primary modes of cancer induction are likely to differ as exposure concentrations decrease below those required to induce lung particle overload, the slope of the dose-response curve is also likely to change. Since a change in slope at low doses cannot be determined from available bioassay data, low-dose extrapolation results in a considerable degree of uncertainty.

In summary, the use of rat data to quantitate human cancer risk at environmental exposures is not recommended.

8.2.5. Epidemiology-Based Estimation of Cancer Potency

The first lung cancer risk estimates based on epidemiologic data were derived by Harris (1983). He 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 excess 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.

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 1900 to 7400. Employing these values, the lifetime 95% upper confidence limits of the risk from exposure to 1 $\mu g/m^3$ DE can be derived, 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. Based upon the exposure estimates of 500 or 125 $\mu g/m^3$ DPM, unit risks of 0.6×10^{-3} and $2 \times 10^{-3}/\mu g/m^3$ 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.

California EPA (1998) derived unit risk estimates for lung cancer, based upon the Garshick et al. (1987) case-control study and the Garshick et al. (1988) cohort study of U.S. railroad workers. A variety of exposure patterns were considered, characterized by two components: the average exposure concentration for the workers as measured by Woskie et al. (1988) and the extent of change from 1959 to1980. The lowest lifetime risk estimate derived was $1.3 \times 10^{-4}/\mu g/m^3$ and the highest was $2.4 \times 10^{-3}/\mu g/m^3$. The geometric mean was $6 \times 10^{-4}/\mu g/m^3$.

Steenland et al. (1998) estimated lung cancer risk of truck drivers on the basis of a case-control study of decedents in the Teamsters Union (Steenland et al., 1990). Retrospective exposure estimates were made starting with a set of 1991 exposure measurements for different job categories and then retrospectively estimating from 1982 to about 1950 using various factors, including diesel vehicle miles traveled and DE engine emission rates per mile. The 1991 job category estimates came from an extensive industrial hygiene survey of elemental carbon (EC) exposures in the trucking industry by Zaebst et al. (1991). Lifetime (through age 75) excess risk of lung cancer death for male truck drivers was calculated with the aid of a cumulative exposure model. Assuming a most likely emissions scenario of 4.5 gm/mile in 1970, and a 45-year exposure to 5 $\mu g/m^3$ of EC beginning at age 20 and ending at age 65, the estimated excess lung cancer risk was determined to be 1.6% (95% CI 0.4%-3.1%).

8.2.6. Suitability of Using 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. 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 (at least as defined in the rat studies) under either occupational or ambient exposure levels. Uncertainty in extrapolating risk 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

concentration of PAHs but lacks an insoluble carbon core. Increased levels of aromatic DNA mortality in Chinese communes burning so-called smoky coal containing high concentrations of polycyclic aromatic hydrocarbons (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 adducts were reported in bus maintenance and terminal workers by Hemminki et al. (1994) and in garage workers and mechanics exposed to DE (Nielsen 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 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, 1988) studies is decreased by the considerable effort exerted to eliminate or reduce such factors, especially smoking. Moreover, meta-analyses by Bhatia et al. (1998) and Lipsett et al. (1999) 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 a reasonable approximation.

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., 1988a). In the only historic study found in which DPM was measured, Heino et al. (1978) reported average concentrations of 2 mg/m^3 in Finnish roundhouses.. Woskie et al. (1988b), 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.

With some of the uncertainties about the dose-response for both railroad workers and truck drivers being actively investigated by EPA, NIOSH, and others, EPA feels that additional in-depth dose-response analysis should await the newer data that are expected during 2000.

8.2.6.1. *Railroad Worker Data*

Although there have been previous efforts to use Garshick's cohort data to conduct a quantitative risk assessment, a consistent dose-response between DE exposure and lung cancer has not been found. Both positive and negative dose-responses have been reported, depending on how the data are analyzed. These conflicting analyses have become a source of continuing debate about estimated cancer risk to humans through DE exposure. Presently, a known mortality under count in the Garshick data is being funded by NIOSH and updated by Garshick. In the near future this updating will likely be reanalyzed by several risk assessing institutions to see if some of the uncertainties have been resolved. For these reasons, EPA will not conduct its own quantitative risk assessment until more information becomes available. This decision should not be construed to imply that the railroad worker studies contain no useful information on lung cancer risk from exposure to DE.

Crump et al. (1991) reported that the relative risk can be positively or negatively related to the duration of exposure depending on how age was controlled in a model. Garshick et al., (1988) reported a positive relationship of relative risk and duration of exposure by modeling age in 1959 as a covariant in an exposure-response model. The positive relationship disappeared when attained age was used instead of age in 1959. This negative dose-response continues to be upheld and further clarified in Crump (1999). California EPA (Cal-EPA, 1998) also found a positive dose-response by using age in 1959 but allowing for an interaction term of age and calendar year in the model. Cal-EPA produced a unit risk estimate in the range of 10^{-3} to 10^{-4}.

A recent special report by HEI (1999) also suggested there was no positive dose-response if a model similar to that used by Cal-EPA included a variable to stratify data into three job categories: clerks, shop workers, and train workers. This observation suggests that one should be cautious in estimating relative risk by taking the ratio of two relative risks calculated from two different job groups (e.g., train workers vs. clerks) because there appear to be some unknown job-category-specific effects operating among these groups.

8.2.6.2. *Teamster Truck Driver Data*

Steenland et al. (1998) is a case-control study of members of the Teamsters Union who died in 1982-1983. Smoking histories were obtained from next of kin. Available data indicate that exposure to workers in the trucking industry in 1990 averaged 2-27 $\mu g/m^3$ of elemental carbon (EC). The exposure information in 1990 was used as a baseline exposure measurement to

reconstruct past exposure (in the period of 1949 to 1983) by assuming that the exposure for workers in different job categories is a function of highway mileages traveled by heavy-duty vehicles, and efficiency of the engine over the years.

Steenland et al. (1998) provide a potentially valuable database for calculating unit risk for DE emissions. The strength of this data set is that the smoking history of workers were obtained to the extent possible. Smoking is especially important in assessing the lung cancer risk due to DE exposure, because smoking has much higher relative risk (or odds ratio) of lung cancer in comparison to that of DEP. For the Steenland et al. (1998) study, the overall (ever-smokers vs. nonsmokers) odds ratio for smoking is about 7.2, which is about fivefold larger than the 1.4 odds ratio of diesel exposure. It is possible that a moderate change of information on smoking and diesel exposure might alter the conclusion and risk estimate.

EPA has noted that Steenland's Teamsters Union truck driver case control study workers had cumulative exposure ranging from 19 to 2440, with the median and 95th percentile respectively of 358 and 754 $\mu g/m^3$-years of EC. These EC levels correspond respectively to 197 and 415 $\mu g/m^3$-years of DE in ambient air, or approximately 3 and 6 $\mu g/m^3$ of DE in ambient air. This information is useful for comparing the exposure of the trucking personnel to animal bioassay exposures and to estimated environmental exposures.

Steenland et al. (1998) indicate that their risk assessment is exploratory because it depends on estimates about unknown past exposures. With the Steenland risk assessment being a recent publication, independent evaluation of the uncertainties have been limited to HEI (1999) and a few interactions with stakeholders. HEI raised questions about the exposure estimates and the selection of controls; EPA has also noted that it may have databases to facilitate the development of improved exposure estimates. EPA and NIOSH are jointly pursuing some of the questions, including the exposure aspects. This work will be ongoing well into 2000.

Give the ongoing review and reanalysis, EPA will not use the Steenland occupational risk assessment to derive equivalent environmental parameters and cancer unit risk estimates until the additional investigation and reanalysis is completed and can be evaluated.

8.3. OBSERVATIONS ABOUT RISK

8.3.1. Perspectives

A decision has been made for this report that, despite the finding that DE is best characterized as highly likely to be a lung cancer hazard, the available data are currently unsuitable to make a confident, quantitative statement about the magnitude of the lung cancer risk attributable to DE at ambient exposure levels. However, the following information is provided to put DE cancer hazard in perspective and to help decision makers and the public make

prudent public health judgments in the absence of a definitive estimate of the upper bound on cancer risk.

The characterization of lung cancer hazard is based on over 20 studies that demonstrate a consistent, positive association between exposure to DE in occupational settings and lung cancer risk. Several meta-analyses have also reported this consistent but relatively low increase in relative risk of about 40%. Notwithstanding the discussion that was previously presented as to why these relative risk estimates and their attendant exposure assessments are uncertain, these positive associations suggest the potential for lung cancer risk at typical, historical occupational exposures. Since epidemiology is a relatively crude tool, we have come to expect that increased risks that are discernable over background cancer mortality in such studies will generally exceed 1×10^{-5} (1 in 100,000) and are often as high as 1×10^{-3} (1 in 1000). Unless the magnitude of the risk is in this range, given typical population sizes and competing biases that tend toward the null, results would be expected to be nonpositive (no statistically significant association). If actual risk were much higher than this ($>10^{-3}$ or 1 in 1000), relative risks would probably be much higher. While ambient average exposures are surely less than workplace exposures today, the margin of exposure for some individuals at the high end (>90 percentile) of the exposed population compared to the lower end of occupational exposures appears to be less than a factor of 10. This means that, when occupational exposures are converted to full day rather than workday and proximity to urban sources is considered, some individuals in the population may be experiencing exposures that are close to or even overlapping the exposures characterized in the Steenland et al. (1998) truckers study. Exposure estimates reported in that study are the focus of additional scrutiny but, based on EPA insights about the exposure review, it seems unlikely that exposure estimates would change by orders of magnitude and significantly alter the perspective just presented. While this perspective falls short of providing a definitive estimate of risk, it should be useful in providing a perspective on potential risk.

In addition, approaches to characterize risk to DE using comparative potency methodologies suggest that upper-bound risks under estimated ambient exposure situations could be in the range of 10^{-4} to 10^{-5} with upper-bound unit risk estimates clustering around 10^{-5} per $\mu g/m^3$ of DEP. While this approach is not amenable to deriving a reliable point estimate of upper-bound lung cancer risk for reasons described in Section 8.2., it does help to put potential risk in perspective because it relies on comparisons with other combustion products (coke oven emissions or cigarette smoke condensate) or from pyrolysis products (roofing tar) for which epidemiologic-based unit risk estimates have been developed.

EPA (1998) also developed a cancer risk estimate using benzo(a)pyrene (B[a]P) as a dosimeter. Pike and Henderson (1981) found good agreement when relating the concentration of (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 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 1/1500 per ng/m^3 B[a]P and a reported B[a]P concentration of 3.9 ng/μg DEP 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 was estimated to be near 1×10^{-5}. The use of B(a)P as a dosimeter provided reasonably good estimates of lung cancer risk for combustion and pyrolysis products of coke ovens, hot pitch, gas production, refining, etc., in spite of the fact that B(*a*)P may constitute a relatively small fraction of the carcinogens present in these emissions. Risk estimates were 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 from those combustion sources, unlike diesel particles, generally lack an insoluble carbon core. As noted in Chapters 3 and 7, adsorption to an insoluble particle core is likely to influence potency of individual organic components because of possible differing elution or activation rates. 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 in detail. While this approach appears to be useful for estimating risk from a variety of related combustion emissions, because these emissions lacked an insoluble core, there is uncertainty.

In the absence of definitive risk estimates in this assessment, the three perspectives just discussed are not to be taken as absolutes, but rather as an outlook. With ongoing investigations regarding the existing key epidemiologic studies, as well as newly started epidemiologic studies focused on diesel exhaust, there are likely to be new future opportunities to consider risk estimation for diesel exhaust.

8.4. SUMMARY OF CANCER DOSE-RESPONSE CONSIDERATIONS

A number of attempts have been made to estimate cancer risk from exposure to DE. The present scientific consensus, however, suggests that animal- and other nonhuman-based risk estimates are too uncertain to extrapolate to humans. Therefore, EPA will focus on the use of epidemiological data to develop quantitative risk assessment. Because some of the uncertainties about the dose-response for both railroad workers and truck drivers can be reduced or better characterized by obtaining additional data within a reasonable time frame, EPA sees no value at this juncture in further teasing the existing data with additional dose-response analysis to unravel some of the uncertainties. With additional investigations underway in two of the richest epidemiology data sets, the railroad worker data base and the Teamster truck driver database,

EPA will await developments before taking additional steps to derive a cancer unit risk for exposure to DE. EPA expects this newer information to be incrementally available throughout 2000.

At this time EPA is not adopting or recommending any cancer unit risk estimate for DE.

While there are uncertainties and controversy with the DE risk estimates that are available from various investigators, this uncertainty can not be resolved with currently available scientific information. The uncertainty about estimating unit cancer risk should not be confused with the inference that DE is "highly likely" to be a human carcinogen and that most would agree that it has produced risk (e.g., a relative risk increase of about 1.4) in some occupational settings. This elevation of risk is of public health concern even though the exposure-lung cancer risk relationship (e.g., unit risk) is uncertain. This concern is further demonstrated when comparing some high end environmental exposure estimates to lower end occupational exposure estimates and realizing that the exposure margin seems to be relatively small. Having information to put the DE cancer hazard into perspective may be useful in the absence of definite risk estimates.

Risk estimates derived from epidemiology studies are preferred for DE and are the pursuit of additional research and analysis.

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9. CHARACTERIZATION OF HEALTH HAZARD AND DOSE-RESPONSE FOR DIESEL ENGINE EXHAUST

9.1. INTRODUCTION

Earlier chapters focused on specific health assessment topics and developed key findings for these topics or provided an overview of relevant background information. This chapter will integrate the key findings about health hazards and dose-response analysis for humans exposed to diesel exhaust (DE). Health hazard characterization and dose-response analysis are two of the four components of risk assessment. A third component, exposure assessment, is not within the scope of this report, though an environmental exposure perspective is included in Section 2.4 to assist in evaluating some aspects of the available toxicological information. The fourth component, a population-based risk characterization for environmental exposures to diesel engine exhaust, is beyond the scope of this document.

For introductory purposes, an overview of themes from 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 suspicions about adverse health effects for DE given a sufficient dose, dose-rate, or cumulative exposure.
- Because DE is a mixture, the choice of a dosimeter for measuring exposure is important; $\mu g/m^3$ of diesel particulate matter (PM) is used as the dosimeter for the entire DE mixture.
- Ambient exposures to DE vary widely depending on the proximity to sources of diesel engine emissions, including on-road vehicles, off-road machinery, railroad locomotives, and ships. Generally speaking rural locations have lower concentrations of DE than do urban areas, and proximity to occupational settings where diesels are in frequent use provides opportunities for even higher exposures. The margin between high end environmental exposures and occupational exposures is of interest.
- Noncancer toxicity: For chronic exposure, there is scanty human but much animal evidence for adverse respiratory effects, such as airway restriction, inflammation, and related measures of pulmonary histopathology. Acute exposure in humans may elicit symptoms of irritation, ranging from annoying or temporarily debilitating symptoms reflecting tissue irritation. An emerging concern is the

possible role of DE in exacerbating or initiating allergenic effects following acute or chronic exposure. The similarity or difference in these DE effects compared with ambient fine particulate matter is of interest.

Carcinogenicity: Occupational epidemiologic studies, using surrogates for DE exposure, show a pattern of increased cancer risk for the lung. Most rat and some mouse inhalation studies show a carcinogenic response in the lung at high test exposures; in the rat these responses occur under conditions of particle overload. Organic components of DE have known or suspected mutagenic/genotoxic and carcinogenic properties. Mode-of-action information provides a framework to evaluate the observed lung cancer responses and judge the confidence in establishing the human hazard potential as well as suggesting the best approach for conducting dose-response analysis and estimation of cancer unit risk.

9.2. WHAT IS DIESEL EXHAUST IN A HEALTH HAZARD ASSESSMENT CONTEXT?

DE is a complex mixture of literally hundreds of components. As reviewed in Chapter 2, the mixture consists of particles and gases. The particulate matter consists of an elemental carbon core particle with hundreds of organic and some inorganic compounds adsorbed to the particle surface. The gaseous fraction is also made up of many organic and multiple inorganic compounds. Some organics and inorganics also exist in a semivolatile state. The elemental carbon core, the particle coating of adsorbed compounds, and the gaseous and semivolatile elements each have constituents with known toxicological properties, and in addition there is a possible aggregate toxicological potential for the whole mixture.

The DE particle fraction is made up of a distribution of particle sizes (e.g., nano/ultrafine particles of 0.005-0.05 μm mean mass aerodynamic diameter), as well as clusters of aggregated particles (e.g., fine particles of 0.05-0.7 μm MMAD) and a small number of larger particles (e.g., coarse size of 1.0-10.0 μm MMAD) (Section 2.6.5). Typically the particles average about 0.2 μm MMAD and have a very large surface area (50-200 m^2/g). Most of the particle mass is in the fine size range, while the majority of the particles are in the nano/ultrafine range. The vast majority of DE particles will be present in a $PM_{2.5}$ fraction of total PM. In any given ambient PM sample, diesel particles may or may not be present, depending on the proximity to a diesel emission source. The diesel particle is crudely distinguishable from other PM by virtue of its elemental carbon core and possibly certain qualitative or quantitative differences in the adsorbed organics. DE may contribute significantly to total ambient PM: for instance, Schauer et al. (1996) reported nationwide diesel contributions to total $PM_{2.5}$ mass of 12.8%-35.7% in several urban California

regions in 1982, whereas the more current Denver area NFRAQS (1998) study showed diesel $PM_{2.5}$ to be 9.7%-10.2% of total $PM_{2.5}$ mass. The U.S. EPA Air Quality Planning and Standards report on air pollutant trends indicates that annual emissions of diesel $PM_{2.5}$ nationwide are 5.7% of the total $PM_{2.5}$ inventory and 21% of the inventory excluding natural and fugitive dust sources.

The diesel particle size distribution is significant for exposure-response purposes because smaller particles have a greater likelihood of being deposited more deeply in the lung than do larger diameter particles. Additionally, smaller particles have a larger surface area per unit of mass and therefore may adsorb and transport more organic compounds into the respiratory system than the same mass of larger particles, and may elicit more of an inflammatory response characteristic or poorly soluble particles (Section 7.4.1). From these circumstances, it would be suspected that DE particles may have a different (e.g., increased) potential for toxicological consequences compared to larger particles of other than DE origin.

The main constituent by weight of the diesel particle is elemental carbon (Section 2.2.6.1). Various studies show the DE particle composition to vary considerably, with the elemental carbon content ranging from 30% to 90% of total mass, with 80% being typical. (For reference, PM from gasoline engine exhaust typically has a much smaller fraction of elemental carbon and a large organic fraction.) The DE inorganics include nitrates, compounds of sulfur, and some carbon monoxide. The DE particle organics include many compounds, a number of which are considered to have a mutagenic and carcinogenic hazard potential for humans (see Table 2-9 for classes of compounds), though the concentrations of the organics are generally low. Many PAHs and PAH derivatives are toxic, especially the nitro-PAHs. Many of the compounds emitted as gases are also potentially carcinogenic or otherwise toxic at some dose, though not necessarily known to be toxic to the lung. These include benzene, 1,3-butadiene, various aldehydes, ethylene dibromide, nitroaromatics, oxides of nitrogen, and sulfur compounds. Additionally, there is evidence that the mixture of organics emitted and as altered in atmospheric transformation provides the chemical species necessary for the formation of free radicals (e.g., reactive oxygen or hydroxyl species formed from certain organics with or without mammalian metabolism); free radicals are known to cause DNA damage in biological systems (Section 7.4.3).

The quantitative physical-chemical composition of any discrete diesel exhaust depends on numerous factors, including operating conditions, heavy-duty versus light-duty engines, engine design, engine age, fuel used, exhaust control technology, and the sampling and measurement system used. Diesel particle measurement in the laboratory under controlled conditions versus sampling in the ambient environment is likely to produce varied results, because the formation of particles is influenced by dilution ratios and conditions of temperature and humidity. These factors mostly affect particle size but may also affect particle composition. The available human

and animal studies were based on engine exhaust representative of engines and conditions at various times since 1980, while some of the epidemiology studies cover exposures from the 1950s through the mid 1980s. This leads to two questions: how the physical-chemical nature of the past exposures compares to present-day exposures, and how applicable the toxicological results generated from the older exposures are to current-day DE exposure-related hazards. These questions frame a risk assessment uncertainty for which there are no definitive answers.

The overwhelming majority of the emission, exposure, and toxicological data uses particle emission mass expressed in units of $\mu g/m^3$ for DE measurement. This was assumed early on by researchers to be a useful dosimeter. At first glance this approach seems to ignore the gaseous component and it does not distinguish between elemental carbon and the accompanying organics.

In Sections 2.2.6 and 2.2.7 an attempt is made to characterize the changes in engine emissions over the years, taking into consideration the lack of consistent and reliable data and the variability of in-use engines. What can be crudely inferred from the available data is that trends in the emission composition over the years have not changed much, qualitatively, though some quantitative changes are discernible in the past 20 years. By analysis, on-road diesel engine particulate emissions were reduced about sixfold, at most 10-fold, on a g/mile basis from 1977 to 1997. Both the elemental carbon and organic content are decreasing. The decrease in organics is mostly a consequence of engine designs that seek to reduce oil consumption. Available research suggests that while most PAH emissions, including nitro-PAHs show a declining trend on a g/mi basis, the overall PAH composition profile has not changed significantly. There is no available evidence that toxicologically significant organic components of DE (e.g., PAHs, PAH derivatives, and nitro-PAHs) have significantly increased or decreased out of proportion to the change in organic mass. Limited particle size measurements suggest that current engine emissions may have higher concentrations of nano/ultrafine particles; however, the methods for measuring these particles are in an early stage of development, and at the moment there is little concrete evidence that modern engines produce greater amounts of <0.05 μm particles than older engines. Given this information and recognizing the extensive use of $\mu g/m^3$ in published research results, $\mu g/m^3$ is used as the dosimeter in this assessment. The best choice of dosimeter and subsequent reduction of uncertainty will only be discernible when there is a better understanding of diesel's toxicological mode of action.

The second question, the applicability of past exposure-toxicological results to present-day exposure scenarios, is not fully answerable and thus remains an area of uncertainty. The observation that there is no particular evidence for a major qualitative change in organic composition, especially for PAHs, and that organics can be viewed as proportional to the particle

mass provides a rationale for the applicability of prior-year assessment findings to more current exposures when $\mu g/m^3$ is used as the dosimeter.

Once diesel emissions are released in the air, they are subject to dispersal, dilution, and chemical and physical transformations (Section 2.3.3). Newly emitted exhaust is termed "fresh" while exhaust more than 1 or 2 days old is referred to as "aged" because of alterations caused by sunlight and other chemical physical conditions of the ambient atmosphere. It is not clear what the overall toxicological consequence of exhaust aging is, because some compounds are altered to more toxic forms while others are made less toxic. For example, PAHs present in fresh emissions may be nitrated by atmospheric NO_3 to form nitro-PAHs, thus adding to the existing burden of nitro-PAHs present in fresh exhaust; alkanes and alkenes may be converted to aldehydes, and oxides of nitrogen to nitric acid. The atmospheric lifetime for some of the transformed compounds ranges from hours to days (Chapter 2, Table 2-9). On the other hand, PAHs present in the gas phase react with hydroxyl radicals present in the ambient air, leading to reduced atmospheric halftimes of the original PAHs. In general, secondary pollutants formed in an aged aerosol mass are more oxidized, and therefore have increased polarity and water solubility. Comprehensive assessment of the health hazards posed by DE would also consider the hazards posed by the atmospheric reaction products, a task that is not directly addressed in this assessment. In terms of environmental and occupational concentrations of DE, most people exposed to DE receive a mixture of both fresh and aged exhaust, with the proportion of fresh exhaust likely related to their proximity to the source of emissions. On the other hand, the DE used in animal bioassays had a high percentage of fresh exhaust.

9.3. NONOCCUPATIONAL AND OCCUPATIONAL EXPOSURE

While a rigorous and comprehensive exposure analysis for DE has not been conducted as part of this assessment, some exposure information from EPA's Office of Mobile Sources has been included in Section 2.4.3 to provide a context for the hazard assessment and dose-response analysis. Nonoccupational exposure to DE occurs worldwide in urban areas, with lesser exposure in rural areas. The concentration of DE constituents in the air will vary within any geographic area based on the number and types of diesel engines (on-road and off-road) in the area, the atmospheric patterns of dispersal, and the proximity of the exposed individual to the diesel emission source. Certain occupational populations can be exposed to much higher levels of DE compared with a majority of the population.

In developing a perspective on human exposure one has to distinguish between airborne concentrations present at any given time versus actual human exposure. Estimates of annually averaged DEP (diesel exhaust particulates) at fixed sites in urban and suburban areas in the 1980s

ranged from approximately 4.4 $\mu g/m^3$ to 11.6 $\mu g/m^3$ from chemical mass balance (CMB) modeling which covers all (on-road and off-road) sources of emission (Section 2.2.4). Modeling shows that an above-average day, representing a high concentration day, may be in the 10 to 22 $\mu g/m^3$ range, and that "hotspots" (near highways, bus depots, or other transportation facilities) may range up to 47 $\mu g/m^3$. In a broader sense, DEP concentrations assessed by CMB for both on-road and off- road at fixed sites in suburban and urban areas range from approximately 1.2 to 3.6 $\mu g/m^3$.

For exposure estimation EPA relies on a Hazardous Air Pollutant Exposure Model which deals with on-road sources only (Section 2.4.4). This model indicates that on an annual basis, the urban population is exposed to levels of DEP from 0.6 to 1.7 $\mu g/m^3$. For more highly exposed individuals in urban areas the range is 0.9 to 4.1 $\mu g/m^3$. These estimates include projections into the 1990s. Those in the population that have outdoor time in proximity to diesel exhaust sources such as highway truck routes are likely to have a higher exposure during the outdoor time, and thus their annual average exposure is somewhat higher than those with lesser outdoor time.

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 (Section 2.2.6.4). ORD's dioxin source emission inventory estimates that 60 g TEQ were emitted from heavy-duty U.S. trucks in 1995. This does not account for other vehicular diesel emissions (e.g., diesel automobiles and other truck categories) or any off-road emissions from the many diesel-powered engines. When the heavy duty truck estimate is compared with total estimated U.S. emissions of 3000 g TEQ for 1995, it appears that the heavy-duty diesel trucks are not a major dioxin source. The human dioxin exposures of concern have been primarily noninhalation exposures associated with human ingestion of certain foods, e.g., beef, vegetables, and dairy products contaminated by dioxin. It is unknown whether heavy-duty truck DE deposition has a local food chain impact.

9.4. HAZARD CHARACTERIZATION

9.4.1. Health Effects Other Than Cancer: Acute Exposures

As reviewed in Chapter 5, the most readily identified acute (e.g., usually single-exposure) 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 or tingling of the extremities. Such symptoms have been reported by individuals exposed to DE on busy city streets or in bus stations, most of which are case reports without an understanding about the possibility of confounding exposures. Recent human and

animal studies also suggest that acute DE exposure episodes may play a role in the development of immunological allergic reactions, possibly resulting in prolonged hypersensitivity to DE and perhaps other ambient contaminants. It is premature to further characterize DE's allergenicity effects until additional information is available.

9.4.2. Effects Other Than Cancer: Chronic Exposure

Based on limited evidence in human occupational studies, but 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, with limited evidence of long-term consequences, whereas in animal studies the onset of symptoms and adverse consequences is more clear and replicable. Current data also identify possible neurological and behavioral effects, though these occur at higher exposure levels than the respiratory effects. Animal studies show a possible high-exposure reproductive effect, but no other reproductive or developmental consequence is identified. Section 5.6 summaries discuss this topic in more depth.

A few human studies in various diesel occupational settings suggest that diesel exposure may impair pulmonary function, as evidenced by increases in respiratory symptoms and some reductions in baseline pulmonary function consistent with restrictive airway disease. Other studies found no particular effects. The methodologic limitations in these studies limit their usefulness in drawing any firmer conclusions (Sections 5.6.1, 5.6.9).

There is a considerable body of animal evidence that clearly correlates DE exposure with pulmonary injury. Short-term animal exposures of high concentrations of diesel 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. Histological studies show 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. 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. While the applicability of rat lung cancer responses to possible human hazard has been questioned, the noncancer rodent responses are thought to be relevant for humans, though the rat is more sensitive than other rodent species and is also suspected to be more sensitive than humans for a number of toxic effects (ILSI, 1998). Because these effects were seen in a wide range of animal species, there is a qualitative basis to believe

that humans could also experience hazard for these effects and may be at risk under some condition of exposure.

Available data limit current efforts to develop hypotheses regarding specific mechanisms or mode of action for DE's respiratory disease impact on humans. The MoA information comes almost entirely from observing rodents, which demonstrate the following: (1) the particle fraction of DE is involved in the etiology of toxicity, though a constituent role for the particle organics and the DE gases cannot be dismissed; (2) similar particle-driven effects occur in different animal species, although the observable onset varies by species; (3) lung injury appears to be mediated by a progressive impairment of normal lung function by invading alveolar macrophages; and (4) it is believed that the adverse effects have a biological threshold, there being no available evidence to the contrary.

Animal studies have also suggested that liver and kidney changes may be occurring at high concentrations, along with some indication of neurotoxic effects and impacts on spermatogenesis. Impaired growth rates have also been observed in animals chronically exposed to DE. However, these effects are seen at exposures higher than the respiratory effects. An assessment focused on determining levels that are likely to be protective for respiratory hazards will be protective for all effects observed to date.

Respirable particles in general have been implicated as etiologic factors in various types of chronic human lung diseases (U.S. EPA, 1996). Ambient 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 majority of DE particle mass is in the low end of the "fine" particle range, and thus contributes to ambient levels of $PM_{2.5}$.

9.4.3. Health Effects Other Than Cancer: Derivation of Inhalation Reference Concentration

A considerable body of evidence provides a basis to infer a noncancer respiratory health hazard following inhalation of DE. On the basis of pulmonary function and histopathological and histochemical effects in rats, a rough estimate can be made concerning what chronic dose/exposure rates of DE (measured in terms of the concentration of diesel PM) cause an adverse effect and which exposures do not; this then is a starting point for estimating protective margins for human exposure. The available human studies, while qualitatively suggestive of possible adverse effects, were inadequate for RfC determination. 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.

The derivation of an RfC for DE is a dose-response approach used by EPA for chronic noncarcinogenic 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. The approach identifies a "critical" effect and related NOAEL; "critical" is defined as the first effect, or its known precursor, that occurs as the dose rate increases. There may be various uncertainties associated with this selection. Second, depending on the critical study, any of several types of uncertainty factors are used to reduce the NOAEL to a level that is thought to be without appreciable hazard to humans. The selection of uncertainty factors is driven by both science and policy considerations focused on uncertainties in the available data or, in some cases, reflecting the absence of data. The resulting RfC is not a bright line (i.e., just above which hazard can be expected); rather, as the human exposure increases beyond the RfC, the margin of protection decreases and the likelihood of hazard is considered to increase.

The DE RfC evaluation closely examined 10 long-term (greater than 1 year) DE inhalation studies in laboratory rats. This is beneficial to the process of RfC determination because the data base on the critical effect has an unusually large number of relevant studies (Chapter 6). The available human studies, as discussed earlier, were qualitatively suggestive of adverse effects but were inadequate for RfC determination. Two key rat studies (Mauderly, 1988; Ishinishi, 1988) were selected because each identified respiratory effects after chronic exposure and provided good information about pulmonary histopathology. The selected studies also spanned a wide range of exposures from 350 to 7000 $\mu g/m^3$, with three exposures in the 350-960 $\mu g/m^3$ range. Human equivalent concentrations (HEC) were calculated from the animal exposure information 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 evolved from a NOAEL of 460 $\mu g/m^3$ (HEC = 155 $\mu g/m^3$) that was related to a LOAEL of 960 $\mu g/m^3$ (HEC = 300 $\mu g/m^3$) (Table 6-2). Although particle overload conditions are thought to occur above 1000 $\mu g/m^3$, the likelihood of lung overload conditions is thought to be minimal at 460 $\mu g/m^3$.

Two principal areas of uncertainty are present in the RfC derivation (Section 6.1.3). As the RfC is based on a chronic animal study, an uncertainty factor of 10 is usually applied for the animal-to-human extrapolation of an effect to account for the possibility that humans may be a more sensitive species than the rodent. This uncertainty is equally parceled ($10^{0.5}$ each) between a

pharmacokinetic (PK) component and a pharmacodynamic (PD) component. As a PK model was used in this assessment to derive the HEC, the uncertainty about the PK component was considered resolved. Application of uncertainty for the PD component was more complex. Although the rat appeared to be clearly more sensitive than humans for the inflammatory responses underlying the observed pulmonary pathology, it was not clear if rats were also more sensitive than humans to those inflammatory processes underlying the observed enhanced allergenicity. In light of this uncertainty, the uncertainty for the PD component is maintained at $10^{0.5}$, which is rounded to 3. A second uncertainty factor of 10 is generally used to account for possible inter-individual variability in sensitivity unless mechanistic or other data suggest otherwise. This uncertainty factor is considered appropriate for the current assessment. The total uncertainty factor is $3 \times 10 = 30$. With 155 $\mu g/m^3$ divided by 30, the resulting RfC is

RfC = 5 $\mu g/m^3$ of diesel particulate matter (DEP).

A comparison of the DE RfC and the $PM_{2.5}$ regulatory standard is not a straightforward endeavor, and caution should be exercised in comparing the output of a health hazard assessment RfC and the product of a science-based regulatory process. Nonetheless, conclusions reached in each of these processes are remarkably similar. EPA's 1997 $PM_{2.5}$ standard is 15 $\mu g/m^3$, as a 3-year average, based on human studies. The noncancer respiratory effects from DE are qualitatively similar to some of those for $PM_{2.5}$. The DE particulates can be a component of ambient $PM_{2.5}$. Compared to ambient $PM_{2.5}$ with no DE component, DE is likely to have a higher proportion of fine and ultrafine particulates and is likely to have a higher or at least a varied content of toxicologically active organic compounds. Although some similarities exist between DE and ambient PM, the differences are potentially significant. A comparison of the DE RfC and the $PM_{2.5}$ standard has considerable complexity.

9.5. CARCINOGENICITY HAZARD CHARACTERIZATION

For inhalation exposure, both human studies and animal bioassays are available for assessment of DE. In fact, both the human and certain aspects of the animal data provide evidence that exposure to DE has the potential to be carcinogenic to humans under some conditions of exposure. Chapter 7 reviews the cancer data in detail. A finding about the hazard potential does not specify the magnitude of the possible impact on an exposed population; this is an issue for dose-response assessment, which is discussed in Chapter 8.

9.5.1. Cancer Hazard

Diesel engine exhaust is "highly likely" to be carcinogenic by the inhalation route of exposure, according to EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment. This hazard is viewed as being applicable to ambient (i.e., environmental) exposures. There is no available evidence to evaluate the hazard from other routes of exposure. The "likely" characterization generally compares with the weight-of-evidence designation "B-1, probable human carcinogen" from the EPA's 1986 Guidelines for Carcinogen Risk Assessment. The overall weight of evidence for DE places it at the upper end of the grouping and hence gives the "highly likely" designation (Section 7.5). The carcinogenic potential of DE is indicated by: (1) consistent association between observed increased lung cancer and DE exposure in certain occupationally exposed workers; (2) induction of lung cancer by whole DE and DE particles in some, but not all, inhalation animal bioassays: (3) induction of cancer from various fractions of the DE mixture, as shown in skin painting, intratracheal, and other noninhalation animal test systems; and (4) the presence of organics on the diesel particles and in the DE gases, some of which have potent mutagenic and carcinogenic properties in their own right, as well as some evidence for the bioavailability of the organics. The mode of action for carcinogenicity in humans is unknown, though it could be suggested that either or both the organics in the DE and the elemental carbon diesel particle contribute to the carcinogenic activity.

Increases in relative risk for lung cancer have been consistently noted in a number of epidemiologic studies, and causality considerations for this observed association are very consistent with DE exposure being causally related to lung cancer (Section 7.5.1) Aggregate estimates from meta-analysis of the statistically increased relative risks for smoking-adjusted studies are 1.33 in one analysis and 1.47 in another (33% or 47% increase in lung cancer above background), though individual studies, such as Steenland et al. (1990), had higher relative risks (e.g., 1.64 and 1.89) for specific groups of workers. Meta-analyses are a tool to evaluate relative risk estimates from multiple compatible studies. Although the approach weights the influence of individual study results in the overall outcome, the analysis does not override uncertainties or limitations in the individual studies. A very recent publication provides yet another pooling of diesel occupational exposure-lung cancer data from two large case-control studies in Germany (Brüske-Holfeld et al., 1999). The aggregate relative risk results were similar to those previously mentioned, with some specific job categories having relative risks greater than 2. This paper will be evaluated further before this assessment is finalized.

The uncertainties with the DE epidemiology data are the typical ones including the possibility that chance, bias or confounding are influencing the observed lung cancer increases (Section 7.2.6.5). The persistence of the lung cancer association in multiple studies and statistical

confidence limits in key studies indicates that chance alone is unlikely to account for the observed relation between DE and lung cancer. A causal interpretation for DE is enhanced when the "Hill" causality criteria are evaulated, noting that an absence or weakness in one or several of the criteria does not prevent a causal finding, though it could be a basis to limit one (Section 7.2.6.6). A weakness in the epidemiology studies showing a positive association is that diesel exposure is inferred from job codes, area descriptions, and the like, which are surrogates for the true underlying exposure. This can lead to nondifferential misclassification of exposure, and while unlikely this might result in a spurious risk estimate in any one study. It is even more unlikely, however, that it would bias a sufficient number of studies in a uniform direction to account for the persistent association observed. Moreover, any bias would likely be toward a lower risk estimate. Not all studies controlled for a tobacco smoke effect. In those studies that did adjust for smoking, there remains a possibility that the adjustment may not be completely effective, and residual confounding by smoking may persist to bias the correlation of DE exposure with lung cancer occurrence. This uncertainty is currently unresolvable.

An inability to satisfactorily minimize all confounding, bias, and exposure uncertainties has resulted in the human evidence being judged not quite adequate to support a finding of causality and characterization of DE as a "known" human carcinogen. Others looking at the same evidence may reach slightly different conclusions as scientific judgment is involved. Cal-EPA, for instance, has judged the epidemiologic evidence to be sufficient to support a causality finding under its criteria. Others, HEI (1995) for example, have argued that human data are consistent in showing weak associations between DE exposure and lung cancer, but that there is insufficient evidence to conclude whether confounding and exposure uncertainties have influenced the association.

While lung cancer has been induced experimentally in rats via inhalation of DE at high exposure concentrations, the data show that the primary factors that are likely to be responsible for lung cancer are high particle concentrations producing a particle overload in the lung, and subsequent induction of persistent inflammatory responses, followed by DNA damage, rapid cell turnover, and eventual lung cancer (Section 7.4.2). This mode of action for lung carcinogenicity in the rat under overload conditions is thought to be unique. It is not known whether humans have a similar response pattern at high exposures, although such a pattern has not been historically observed. Overload inflammatory responses are not seen at rat test exposures below 1000 $\mu g/m^3$ (estimated HEC 300-350 $\mu g/m^3$), but lung impacts still occur under the nonoverload condition. Uncertainty remains as to whether induction of inflammatory responses or other forms of lung injury in humans will lead to lung cancer. Therefore, there are insufficient data to conclude that the rat response is completely irrelevant for a human hazard characterization. The

high-exposure-related rat lung cancer responses, however, are unsuitable for estimating risk at lower environmental levels of exposure in humans.

Generally, rats showed significant increases in lung tumors beginning at exposures of >2200 $\mu g/m^3$ (HEC is approximately 700-900 $\mu g/m^3$). These exposure levels clearly represent lung overload conditions for the rat. In addition, these human equivalent exposure concentrations are significantly higher than those found in the human occupational studies discussed in Chapter 7. These range from about 3 $\mu g/m^3$ as an environmental equivalent calculated from the Teamster's Union truck study (Steenland et al., 1998, Section 8.3.8.2) to 141-192 $\mu g/m^3$ (and possibly up to 500 $\mu g/m^3$), which is reported as an occupational level in the railroad worker study (Woskie et al., 1988b and others). These reported levels overlap with, but range significantly higher than, nationwide ambient continuous exposure estimates for humans of 0.6-4.1$\mu g/m^3$, not counting hotspots (Section 2.4.3).

Organic extracts of DE particles have been shown to induce tumors in mice, both by skin painting, and subcutaneous injection, and to be mutagenic in several test systems. Additionally, a number of PAHs and nitro-PAHs present on diesel particles as well as in the vapor phase are known to be mutagenic and/or carcinogenic. As discussed in Section 7.3.2, filtered DE (i.e., exposure to DE gases) does not produce a lung tumor response in rats. Intratracheal studies (Section 7.3.4) show that DE particles with and without organics elicit a lung cancer response, as does a pure elemental carbon particle, carbon black, with a modestly higher response for the whole DE particle. Also, four- to seven-ring PAHs are shown to be a particularly potent fraction of the organic extracts.

The plausibility of an environmental lung cancer hazard from DE by inhalation exposure is supported by findings contained in this assessment: (1) that mutagenic and tumor initiating carcinogens are present in small quantities in the DE organic mixture; (2) that some bioavailability of the organics is expected and that deposited particulates seem to have much longer residence times in humans than in animal species. This provides an extended opportunity for elution, metabolism if needed, and uptake of the organics. These organics include many well characterized mutagens and carcinogens; and (3) that there may be a relatively small margin of exposure between higher end environmental exposures and some occupational levels in studies where statistically increased aggregate relative risks in the range of 1.33 to 1.47 are seen (e.g., exposure estimates for some truck drivers could be overlapping some environmental estimates).

Overall, the evidence for a likely human lung cancer hazard by inhalation is persuasive, even though, in the absence of complete data, inferences and thus uncertainties are involved. Some of the key uncertainties include: (1) methodologic limitations inherent in epidemiologic studies, as well as a lack of reliable historical exposure data for occupationally exposed cohorts,

(2) uncertainties regarding the extent of bioavailability of organic compounds present on diesel particles and their impact on the carcinogenic process, and (3) other uncertainties regarding the mode of action of DE on lung cancer in humans.

The epidemiologic evidence for DE being associated with other forms of cancer is inconclusive.

9.6. CANCER DOSE-RESPONSE ASSESSMENT

Cancer dose-response assessment describes what is known about the relationship of exposure/dose to a cancer response (e.g., lung cancer) and how the response might change with dose within the range of empirical observations. It also evaluates the applicability of this relationship to human low-exposure circumstances. The low-exposure aspects are approached by extrapolation, if appropriate, from an observable response range to lower exposure/dose levels, such as ambient levels of interest. Key choices in dose-response assessment are influenced by epidemiologic and toxicologic data and informed by reasoning about the possible mode(s) of action. In the absence of such information, standard assumptions (i.e., defaults) are used, many of which are conservative toward public health protection. Chapter 8 contains a more detailed review of dose-response issues.

Human data are preferred as a starting point for DE dose-response assessment, one purpose of which would be to estimate cancer potency (i.e., cancer unit risk). Unit risk is the estimated cancer risk at 1 $\mu g/m^3$ of exposure for a lifetime; in this case, $\mu g/m^3$ of DE particulate matter from a continuous 70-year exposure. Unit risk derivation procedures and specifications are defined in EPA's risk assessment guidance.

The overall challenge with DE is to judge the uncertainties in the dose-response analysis, given available data, and to decide whether to proceed. If the analysis is carried out, it is important to decide what certainties/uncertainties to ascribe to any resulting output of the analysis and follow-on unit risk derivation.

The mode of action (MoA) for humans is unknown, and the presumed MoA for rats does not justify using rat lung tumor data to estimate low-exposure cancer risk for humans. This report concludes that a role for organic mutagenic/genotoxic constituents of DE as well as a role for particles is plausible, recognizing that the relative contributions of each may vary with dose-exposure. With organics thought to be in relative proportion to the mass of particulates, the use of $\mu g/m^3$ of DE particles as the dosimeter is feasible. With no clear indication that key organic components have changed disproportionately to total organics over the years (Section 2.5), the use of toxicological results based on older engine exposures to predict current-day hazards is also feasible, though uncertainty exists.

Section 8.2 reviews a number of past attempts to estimate diesel cancer potency (i.e., unit cancer risk) using epidemiology data, rat data, and comparative potency approaches. With the rat estimates now being thought unsuitable, the comparative potency-based estimates having limitations and thus being uncertain, and the epidemiology-based estimates having outstanding issues and questions to be resolved, these historical risk estimates lack a consensus of support. With ongoing investigations to update mortality in the Garshick railroad worker study and additional review and analysis of the Steenland et al. (1998) study underway, the Agency has determined that there is no scientific support for further analysis of the existing epidemiologic data until some newer information is available. Additional information is expected over the next few years.

A decision has been made in this assessment that, despite the finding that DE is best characterized as highly likely to be a lung cancer hazard, the available data are currently unsuitable to make a confident quantitative statement about the magnitude of the lung cancer risk attributable to DE at ambient exposure levels. Therefore, this assessment does not adopt or recommend a specific cancer unit risk estimate for DE. However, information is provided in Section 8.3 to put DE cancer hazard in perspective and to assist decisionmakers and the public to make prudent public health judgments in the absence of a definitive estimate of the upper bound on cancer risk. This perspective is based on the consistent observations of a relatively low (~40%) increase in relative risk and the power of epidemiologic studies to detect low levels of absolute risk. In addition, Section 8.2 describes the use of historic approaches that consider comparative potency to inform the perspective. This discussion leads to the conclusion that the available science can support a position that, if one accepts the conclusion that DE has human carcinogenic potential, risks may be in the range of regulatory interest ($>10^{-6}$ or 1 in 1 million), but that they are not likely to exceed levels that often result in immediate regulatory action ($>10^{-3}$ or 1 in 1000). The Agency does not believe that the current data support a more precise perspective.

9.7. SUSCEPTIBLE SUBGROUPS

The hazards previously characterized, i.e., acute and chronic effects, are assumed to be possible consequences in individuals of average health and in their adult years. There is no DE-specific information that provides direct insight to the question of variable susceptibility within the population. Default approaches to account for uncertainty in inter-individual susceptibility have been included in the derivation of the RfC. Individuals with preexisting lung burdens of particulates may have less of a margin of safety from DE particulate-driven hazards than might be inferred from incremental DE exposure analysis, although this cannot be quantified. DE exposure

could be additive to many other daily or lifetime exposures to organics and PM. 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 could be more susceptible to adverse impacts from DE exposure. Although there is no information from studies of DE, infants and children could have a greater susceptibility to the acute/chronic toxicity of DE because they have greater ventilatory frequency, resulting in greater respiratory tract particle deposition (U.S. EPA, 1996b). The issue of DE impacts on allergenicity and potential onset and exacerbation of childhood asthma is being actively investigated, but firm conclusions await peer review and publication of ongoing work.

Another aspect of differential susceptibility involves subgroups that may receive additional exposure to DE because of their proximity to DE sources. Earlier it was mentioned that those having outside time in their daily routine and being near a diesel emission source would likely receive more exposure than others in the population. The highest exposed are most likely the occupational subgroups whose job brings them very close to diesel emission sources (e.g., trucking industry, machinery operations, engine mechanics, some types of transit operations, railroads, etc.).

9.8. REFERENCES

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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			- (51 ng/m^3)	
Benzo(b)fluoranthene	5.3				
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

Models for Calculating Lung Burdens

B.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 15-30 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 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 that facilitate this removal: (a) mechanical clearance, provided by mucociliary transport in the ciliated conducting airways as well as macrophage phagocytosis and migration in the nonciliated airways; and (b) clearance by dissolution. As 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.

B.2. PARTICLE MODEL

To develop a mathematical model that simulates the deposition and clearance of DEPs in the lung, an appropriate model for diesel particles must be introduced. For the deposition study, we employed an equivalent sphere model developed by Yu and Xu (1987) to simulate the dynamics and deposition of DEPs in the respiratory tract by various 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, which are slowly cleared from the lung; and (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 are not known. It is possible that the fast phase consists of organics that are leached out primarily by diffusion mechanisms while the slow phase might include any or all of the following components: (a) organics that are "loosened" before they are released, (b) organics that have become intercalated in the carbon core and whose release is thus impeded, (c) organics that are associated for longer periods of time because of hydrophobic interaction with other organic-phase materials, (d) organics that have been ingested by macrophages and as a result effectively remain in the lung for a longer period of time because of metabolism by the macrophage (metabolites formed may interact with other cellular components), and (e) organics that 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 does not specifically imply the actual nature of the organics adsorbed on the carbonaceous core; 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).

B.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), as shown in Figure B-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

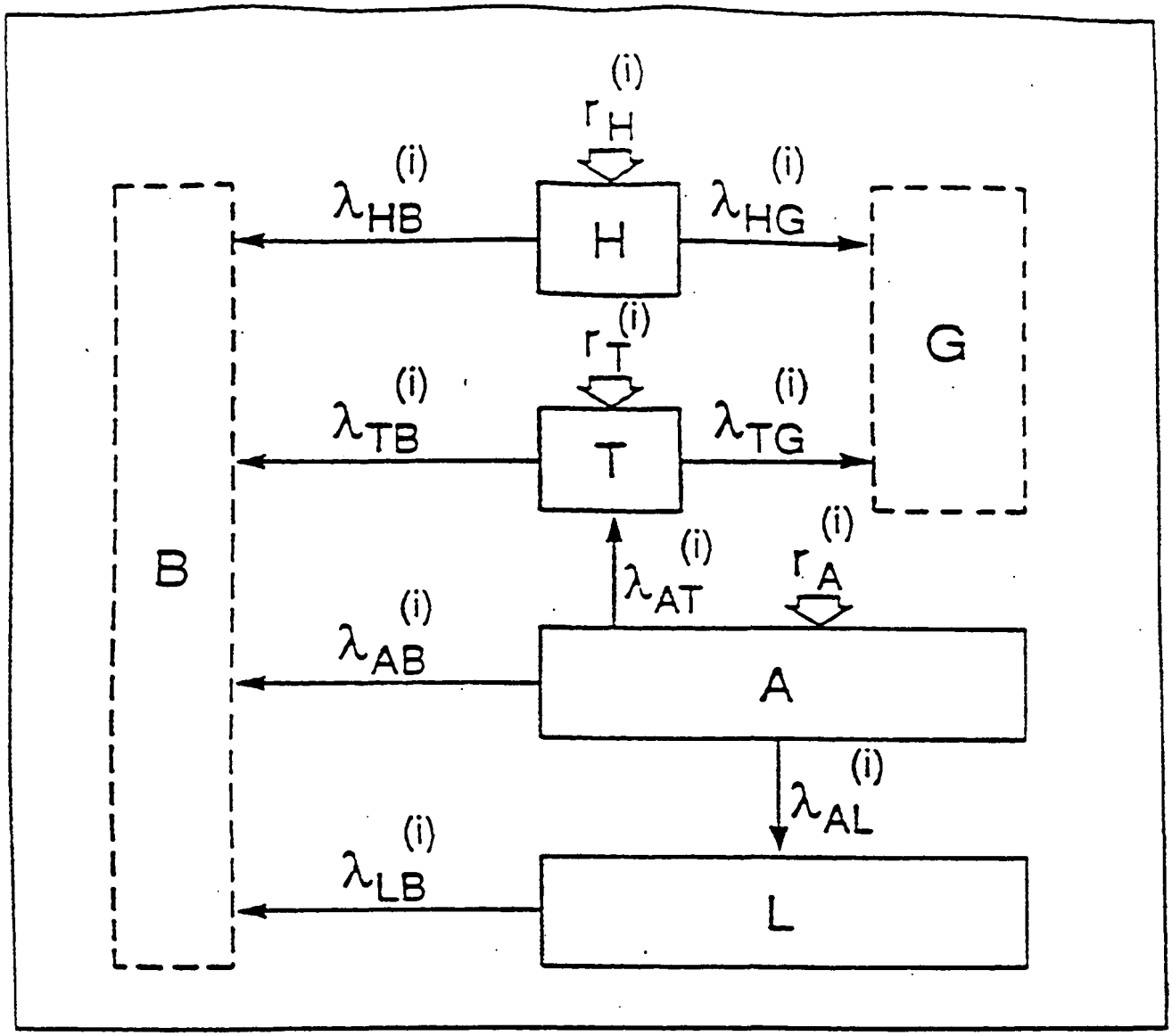

Figure B-1. Compartmental model of DEP retention.

important 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 B-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

$$r_H^{(i)} = f_i\, r_H \quad , \tag{B-1}$$

$$r_T^{(i)} = f_i\, r_T \quad , \tag{B-2}$$

$$r_A^{(i)} = f_i\, r_A \quad , \tag{B-3}$$

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:

$$r_H = c(TV)(RF)(DF)_H \quad , \tag{B-4}$$

$$r_T = c(TV)(RF)(DF)_T \quad , \tag{B-5}$$

$$r_A = c(TV)(RF)(DF)_A \quad . \tag{B-6}$$

In Equations B-4 to B-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).

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)

$$\frac{dm_H^{(i)}}{dt} = r_H^{(i)} - \lambda_{HG}^{(i)} m_H^{(i)} - \lambda_{HB}^{(i)} m_H^{(i)} \quad , \tag{B-7}$$

Tracheobronchial (T)

$$\frac{dm_T^{(i)}}{dt} = r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{TG}^{(i)} m_T^{(i)} - \lambda_{TB}^{(i)} m_T^{(i)} \quad , \tag{B-8}$$

Alveolar (A)

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{AB}^{(i)} m_A^{(i)} \quad , \tag{B-9}$$

Lymph nodes (L)

$$\frac{dm_L^{(i)}}{dt} = \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{LB}^{(i)} m_L^{(i)} \quad . \tag{B-10}$$

Equation B-9 may also be written as

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_A^{(i)} m_A^{(i)} \quad , \tag{B-11}$$

where

$$\lambda_A^{(i)} = \lambda_{AT}^{(i)} + \lambda_{AL}^{(i)} + \lambda_{AB}^{(i)} \quad . \tag{B-12}$$

is the total clearance rate of material component i from the alveolar compartment. In Equations B-7 to B-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

$$m_X = m_X^{(1)} + m_X^{(2)} + m_X^{(3)} \tag{B-13}$$

The lung burdens of diesel soot (core) and organics are defined, respectively, as

$$m_{Lung}^{(1)} = m_T^{(1)} + m_A^{(1)} \quad , \tag{B-14}$$

and

$$m_{Lung}^{(2)+(3)} = m_T^{(2)} + m_A^{(2)} + m_T^{(3)} + m_A^{(3)} \quad . \tag{B-15}$$

Because the clearance of diesel soot from compartment T is much faster than from compartment A, $m_T^{(i)} < m_A^{(i)}$ a short time after exposure, Equation B-14 leads to

$$m_{Lung}^{(1)} \cong m_A^{(1)} \quad . \tag{B-16}$$

Solution to Equations B-7 to B-10 can be obtained once all the transport rates $\lambda_{XY}^{(i)}$ are known. When $\lambda_{XY}^{(i)}$ are constant, which is the case in linear kinetics, Equations B-7 to B-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 B-7 to B-10 can be determined directly from experimental data on lung and lymph node burdens, and $\lambda_{AT}^{(i)}$ and $\lambda_{AB}^{(i)}$ from Equation B-12.

B.4. SOLUTIONS TO KINETIC EQUATIONS

Equation B-11 is a nonlinear differential equation of $m_A^{(i)}$ with known function of $\lambda_A^{(i)}$. For diesel soot, this equation becomes

$$\frac{dm_A^{(1)}}{dt} = r_A^{(1)} - \lambda_A^{(1)}(m_A)m_A^{(1)} \quad . \tag{B-17}$$

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 1%) after a long exposure, and we may consider $\lambda_A^{(1)}$ as a function of $m_A^{(1)}$ alone. Equation B-17 is then reduced to a differential equation with $m_A^{(1)}$ the only dependent variable.

The general solution to Equation B-17 for constant $r_A^{(1)}$ at any time, t, can be obtained by the separation of variables to give

$$\int_0^{m_A^{(1)}} \frac{dm_A^{(1)}}{r_A^{(1)} - \lambda_A^{(1)}m_A^{(1)}} = t \quad . \tag{B-18}$$

If $r_A^{(1)}$ is an arbitrary function of t, Equation B-17 needs to be solved numerically such as by a Runge-Kutta method. Once $m_A^{(1)}$ is found, the other kinetic equations B-7 to B-10 for both diesel soot and the particle-associated organics can be solved readily, as 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)

$$m_H^{(i)} = r_H^{(i)}/\lambda_H^{(i)} + (m_{H0}^{(i)} - r_H^{(i)}/\lambda_H^{(i)}) \exp(-\lambda_H^{(i)} t) \tag{B-19}$$

$$\textit{where} \quad \lambda_H^{(i)} = \lambda_{HG}^{(i)} + \lambda_{HB}^{(i)} \tag{B-20}$$

Tracheobronchial (T)

$$m_T^{(i)} = \exp(-\lambda_T^{(i)} t) \int_0^t (r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)}) \exp(\lambda_{AT}^{(i)} t)\, dt + m_{T0}^{(i)} \quad \text{(B-21)}$$

$$\text{where} \quad \lambda_T^{(i)} = \lambda_{TG}^{(i)} + \lambda_{TB}^{(i)} \quad \text{(B-22)}$$

Lymph nodes (L)

$$m_L^{(i)} = \exp(-\lambda_{LB}^{(i)} t) \int_0^t \lambda_{AL}^{(i)} m_A^{(i)} \exp(\lambda_{LB}^{(i)})\, dt + m_{L0}^{(i)}) \quad \text{(B-23)}$$

In Equations B-19 to B-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 $(DF)_A$ $r^{(DF)}_H$, $r^{(DF)}_T$ and $r^{(DF)}_A$ as well as the values of $\lambda_{XY}^{(i)}$ in the compartmental lung model are presented.

B.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

$$\frac{d_e}{d_a} = \phi^{-1/2}\left(\frac{C_a}{C_e}\right)^{1/2}\left(\frac{\zeta}{\zeta_o}\right)^{1/2} \quad \text{(B-24)}$$

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:

$$C_x = 1 + 2\frac{\lambda}{d_x}\left[1.257 + 0.4 \exp\left(-\left(\frac{0.55 d_x}{\lambda}\right)\right)\right] \quad \text{(B-25)}$$

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 B-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.

B.5.1. Determination of $(DF)_H$

Particle deposition in the naso- or oropharyngeal 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 by 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:

$$(DF)_{H,\ in} = 0,\ for\ d_a^2 \leq 3000 \quad \text{(B-26)}$$

$$(DF)_{H,\ in} = -1.117 + 0.324\ \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \quad \text{(B-27)}$$

$$(DF)_{H,\ ex} = 0, \quad \text{(B-28)}$$

and for nose breathing:

$$(DF)_{H,\ in} = -0.014 + 0.023\ \log(d_a^2 Q),\ for\ d_a^2 Q \leq 337 \quad \text{(B-29)}$$

$$(DF)_{H,\ in} = -0.959 + 0.397\ \log(d_a^2 Q),\ for\ d_a^2 Q > 337 \quad \text{(B-30)}$$

$$(DF)_{H,\ ex} = 0.003 + 0.033\ \log(d_a^2 Q),\ for\ d_a^2 Q \leq 215 \quad \text{(B-31)}$$

$$(DF)_{H,\ ex} = -0.851 + 0.399\ \log(d_a^2 Q),\ for\ d_a^2 Q > 215 \quad \text{(B-32)}$$

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 B-26 through B-32 are transformed into the following form:

For mouth breathing:

$$(DF)_{H,\ in} = 0,\ for\ d_a^2Q \le 3000 \quad \text{(B-33)}$$

$$(DF)_{H,\ in} = -1.117 + 0.972\ logK + 0.324\ \log(d_a^2Q),\ for\ d_a^2Q > 3000 \quad \text{(B-34)}$$

$$(DF)_{H,\ ex} = 0. \quad \text{(B-35)}$$

and for nose breathing:

$$(DF)_{H,\ in} = -\ 0.014 + 0.690\ \log\ K + 0.023\ \log(d_a^2Q),\ for\ d_a^2Q \le 337 \quad \text{(B-36)}$$

$$(DF)_{H,\ in} = -0.959 + 1.191\ \log\ K + 0.397\ \log\ (d_a^2Q),\ \text{for}\ d_a^2Q > 337 \quad \text{(B-37)}$$

$$(DF)_{H,\ ex} = 0.003 + 0.099\ \log\ K + 0.033\ \log(d_a^2Q),\ \text{for}\ d_a^2Q \le 215 \quad \text{(B-38)}$$

$$(DF)_{H,\ ex} = 0.851 + 1.197\ \log\ K + 0.399\ \log(d_a^2Q),\ \text{for}\ d_a^2Q > 215 \quad \text{(B-39)}$$

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:

$$(DF)_{H,\ in} = (DF)_{H,\ ex} = 0.046 + 0.009\ \log(d_a^2Q),\ \text{for}\ d_a^2Q \le 13.33 \quad \text{(B-40)}$$

$$(DF)_{H,\ in} = (DF)_{H,\ ex} = -0.522 + 0.514\ \log(d_a^2Q),\ \text{for}\ d_a^2Q > 13.33 \quad \text{(B-41)}$$

B.5.2. Determination of $(DF)_T$ and $(DF)_A$

The deposition model adopted for DEPs is the one previously developed for monodisperse (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 B-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.

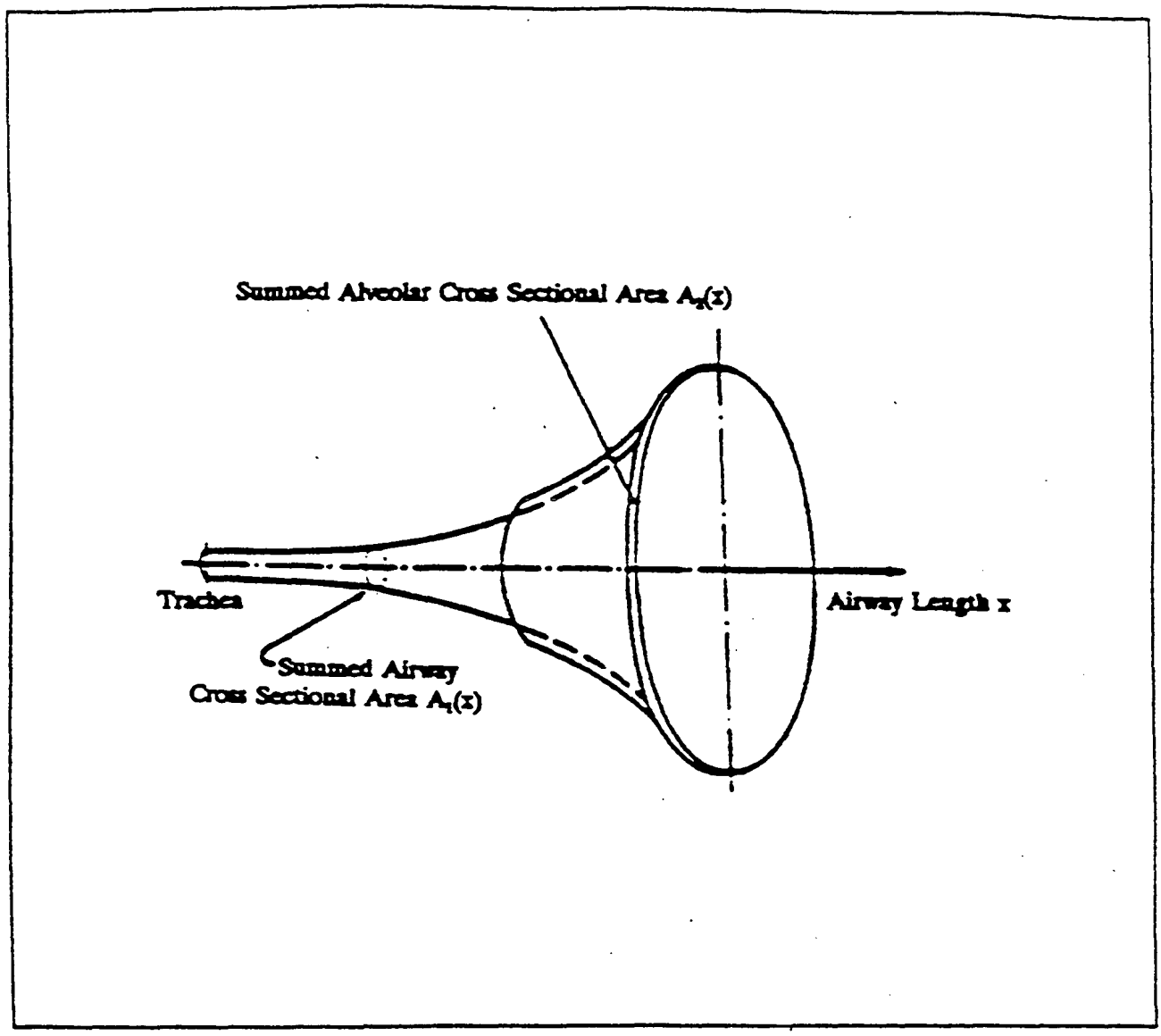

Figure B-2. Trumpet model of lung airways.

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:

$$\beta(A_1 + A_2)\frac{\partial c}{\partial x} + Q\frac{\partial c}{\partial x} = -Qc\eta \qquad \text{(B-42)}$$

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:

$$\beta = 1 + \frac{V_t}{V_l} \tag{B-43}$$

and Q is the air flow rate, varying with x and t according to the relation

$$\frac{Q}{Q_o} = 1 - \frac{V_x}{V_l} \tag{B-44}$$

where Q_0 is the air flow rate at x = 0. In Equations B-43 and B-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 B-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

$$DF = \int_{t_1}^{t_2} \int_{x_1}^{x_2} Qc\eta dx dt \tag{B-45}$$

For diesel particles, η is the sum of those due to the individual deposition mechanisms described above, i.e.,

$$\eta = \eta_I + \eta_S + \eta_P + \eta_D \tag{B-46}$$

where η_I, η_S, η_P, and η_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:

$$\eta_I = \frac{0.768}{L}(St)\theta. \tag{B-47}$$

$$\eta_S = \frac{2}{\pi L}[2\epsilon\sqrt{1 - \epsilon^{(2/3)}} - \epsilon^{1/3}\sqrt{1 - \epsilon^{2/3}} + \sin^{-1}\epsilon^{1/3}] \tag{B-48}$$

$$\eta_P = \frac{4}{3\pi L}(\Gamma - \frac{\Gamma^3}{32}) \tag{B-49}$$

$$\eta_D = \frac{1}{L}[1 - 0.819\exp(-14.63\Delta) - 0.0976\exp(-89.22\Delta) - 0.0325\exp(-228\Delta) - 0.0509\exp(-125\Delta^{2/3})] \quad \text{(B-50)}$$

sfor Reynolds numbers of the flow smaller than 2000, and

$$\eta_D = \frac{4}{L}\Delta^{1/2}(1 - 0.444\Delta^{1/2}) \quad \text{(B-51)}$$

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 $D = C_c kT/(3\pi\mu d_e)$ 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:

$$E = 1 - (1 - E_S)(1 - E_D) \quad . \quad \text{(B-52)}$$

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

$$1 - \sum_{i=1}^{3} \frac{4}{\alpha_i} \exp(-\alpha_i^2\tau_D)(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2}) \exp\left[- \frac{4\tau_D^{1/2}}{\pi^{1/2}(1 - \sum_{i=}^{3}} \right. \quad \text{(B-53)}$$

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:

$$J_o(\alpha) = 0 \quad . \quad \text{(B-54)}$$

in which J_o is the Bessel function of the zeroth order, and:

$$E_S = 1.1094\tau_S - 0.1604\tau_S^2, \text{ for } 0 < \tau_S \leq 1. \quad \text{(B-55)}$$

and

$$E_S = 1 - 0.0069\tau_S^{-1} - 0.0859\tau_S^{-2} - 0.0582\tau_S^{-3}, \text{ for } \tau_S > 1, \quad \text{(B-56)}$$

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.

B.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.

B.5.3.1. *Lung Model for Rats*

Morphometric data on the lung airways of rats were reported by Schum and Yeh (1979). Table B-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.

B.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 B-2 presents the morphometric data of the airways of Weibel's model adjusted to a total lung volume of 3000 cm^3.

Table B-1. Lung model for rats at total lung capacity

Generation number	Number of airways	Length (cm)	Diameter (cm)	Accumulative volume[a] (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[b]	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]Including the attached alveoli volume (number of alveoli = 3×10^7, alveolar diameter = 0.0086 cm).
[b]Terminal bronchioles.

Table B-2. Lung model by Weibel (1963) adjusted to 3000 cm^3 lung volume

Generation number	Number of airways	Length (cm)	Diameter (cm)	Accumulative volume[a] (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[b]	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]Including the attached alveoli volume (number of alveoli = 3×10^8, alveolar diameter = 0.0288 cm).
[b]Terminal bronchioles.

B.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.

B.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

$$N_i(t) = 2^i, \qquad \text{for } 0 \le i \le 20 \tag{B-57}$$

$$\begin{cases} N_{21}(t)=N_r(t), \\ N_{22}(t)=N_{23}(t)=0. \end{cases} \quad \text{for } N_r(t) \le 2^{21} \tag{B-58}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = N_r(t) - 2^{21}, \\ N_{23}(t) = 0, \end{cases} \quad \text{for } 2^{21} < N_r(t) \le 2^{22} \tag{B-59}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = 2^{22}, \\ N_{23}(t) = N_r(t) - 2^{21} - 2^{22} \end{cases} \quad \textit{for } N_r(t) > 2^{21} + 2^{22}, \tag{B-60}$$

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

$$N_r(t) = \begin{cases} 2.036 \times 10^7(1 - 0.926e^{-0.15t}), & t \le 8 \\ 1.468 \times 10^7, & t > 8 \end{cases} \tag{B-61}$$

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 B-58 to B-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:

$$N_A(t) = 2.985 \times 10^8(1 - 0.919e^{-0.45t}) \tag{B-62}$$

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.

B.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 4800 cm^3 for an adult (25 years old), follows the equation

$$LV(t) = 0.959 \times 10^5(1 - 0.998e^{-0.002t}) \quad (cm^3). \tag{B-63}$$

The growth patterns of the bronchial airways are determined by the following equations

$$D_i(t) - D_{iw} = \alpha_i[H(t) - H(25)], \tag{B-64}$$

$$L_i(t) - L_{iw} = \beta_i[H(t) - H(25)], \tag{B-65}$$

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

$$\alpha_i = 3.26 \times 10^{-2}\exp[-1.183\ (i+1)^{0.5}] \tag{B-66}$$

$$\beta_i = 1.05 \times 10^{-6}\ \exp\ [10.1]\ (i+1)^{-0.2}] \tag{B-67}$$

and H(t) is the body height, which varies with age t in the form

$$H(t) = 1.82 \times 10^2(1 - 0.725e^{-0.14t})\ (cm). \tag{B-68}$$

For the growth patterns of the airways in the alveolar region, it is assumed that

$$\frac{D_i}{D_{iw}} = \frac{L_i}{L_{iw}} = \frac{D_a}{D_{aw}} = f(t), \quad for\ 17 \le i \le 23 \tag{B-69}$$

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

$$f(t) = \sqrt[3]{\frac{\{LV(t) - \sum_{i=0}^{16} \frac{\pi}{4} D_i^2(t)\, L_i(t) N_i(t)\}}{\{\sum_{i=17}^{23} \frac{\pi}{4} D_{inv}^2 L_{inv} N_i(t) + \frac{5\pi}{36} D_{aw}^3 N_A(t)\}}} \quad \text{(B-70)}$$

B.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:

$$\lambda_{HG}^{(i)} = 1.73 \quad (i = 1,2,3) \quad \text{(B-71)}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(i)} = 0.00018 \quad \text{(B-72)}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \quad \text{(B-73)}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \quad \text{(B-74)}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \quad \text{(B-75)}$$

$$\lambda_{AL}^{(1)} = 0.00068\ [1 - \exp(-0.046 m_A^{1.62})] \quad \text{(B-76)}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4}\, \lambda_{AB}^{(i)} \qquad (i = 2,3) \quad \text{(B-77)}$$

$$\lambda_{AT}^{(i)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) \quad (i = 1,2,3) \quad \text{(B-78)}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AT}^{(1)} + \lambda_{AB}^{(1)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00086 \quad \text{(B-79)}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_a^{1.62}) + 0.0161 \quad \text{(B-80)}$$

$$\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) + 15.7 \quad \text{(B-81)}$$

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 those 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 B-79 that $\lambda_A^{(1)}$ for rats can be written in the form

$$\lambda_A^{(1)} = a \exp(-bm_A^c) + d \qquad \text{(B-82)}$$

where a, b, c, and d are constants. The right-hand side of Equation B-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 is 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

$$\lambda_A^{(1)} = \frac{a}{P} \exp[-b(m_A/S)^c] + d \qquad \text{(B-83)}$$

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 B-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 B-3.

Table B-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

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:

$$\lambda_{HG}^{(1)} = 1.73 \quad (i = 1,2,3) \tag{B-84}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(1)} = 0.00018 \tag{B-85}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \tag{B-86}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \tag{B-87}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{B-88}$$

$$\lambda_{AL}^{(1)} = 0.00068 \{1 - 0.0694 \exp[-0.046(m_A/S)^{1.62}]\} \tag{B-89}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4} \lambda_{AB}^{(i)} \qquad (i = 2, 3) \tag{B-90}$$

$$\lambda_{AT}^{(i)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} \quad (i = 1, 2, 3) \tag{B-91}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AB}^{(1)} + \lambda_{AT}^{(1)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}]\} + 0.00086 \tag{B-92}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.0694\{0.012 \exp[-0.11(m_A/A)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} + 0.016 \tag{B-93}$$

(B-94)

B.7. RESULTS

B.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 compared 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.

$$\text{Minute volume} = 0.9W \ (cm^3/min) \tag{B-95}$$

$$\text{Respiratory frequency} = 475W^{-0.3} \ (1/min) \tag{B-96}$$

where W is the body weight (in grams) as determined from the equation

$$W = 5+537T/(100+T), \text{ for } T \geq 56 \text{ days} \qquad \text{(B-97)}$$

in which T is the age of the rat measured in days.

Equation B-95 was obtained from the data of Mauderly (1986) for rats ranging in age from 3 mo to 2 years old; Equation B-96 was obtained from the data of Strom et al. (1988); and Equation B-97 was determined from the best fit of the experimental deposition data. Figures B-3 and B-4 show the calculated lung burden of diesel soot ($m_A^{(1)} + m_A^{(2)}$) 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; exposure 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 B-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 B-5 shows that the calculated retention is in excellent agreement with the experimental data obtained by Sun et al. (1984).

B.7.2. Predicted Burdens in Humans

Selected results of lung burden predictions in humans are shown in Figures B-6 to B-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 B-6 and B-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. Because of differences in

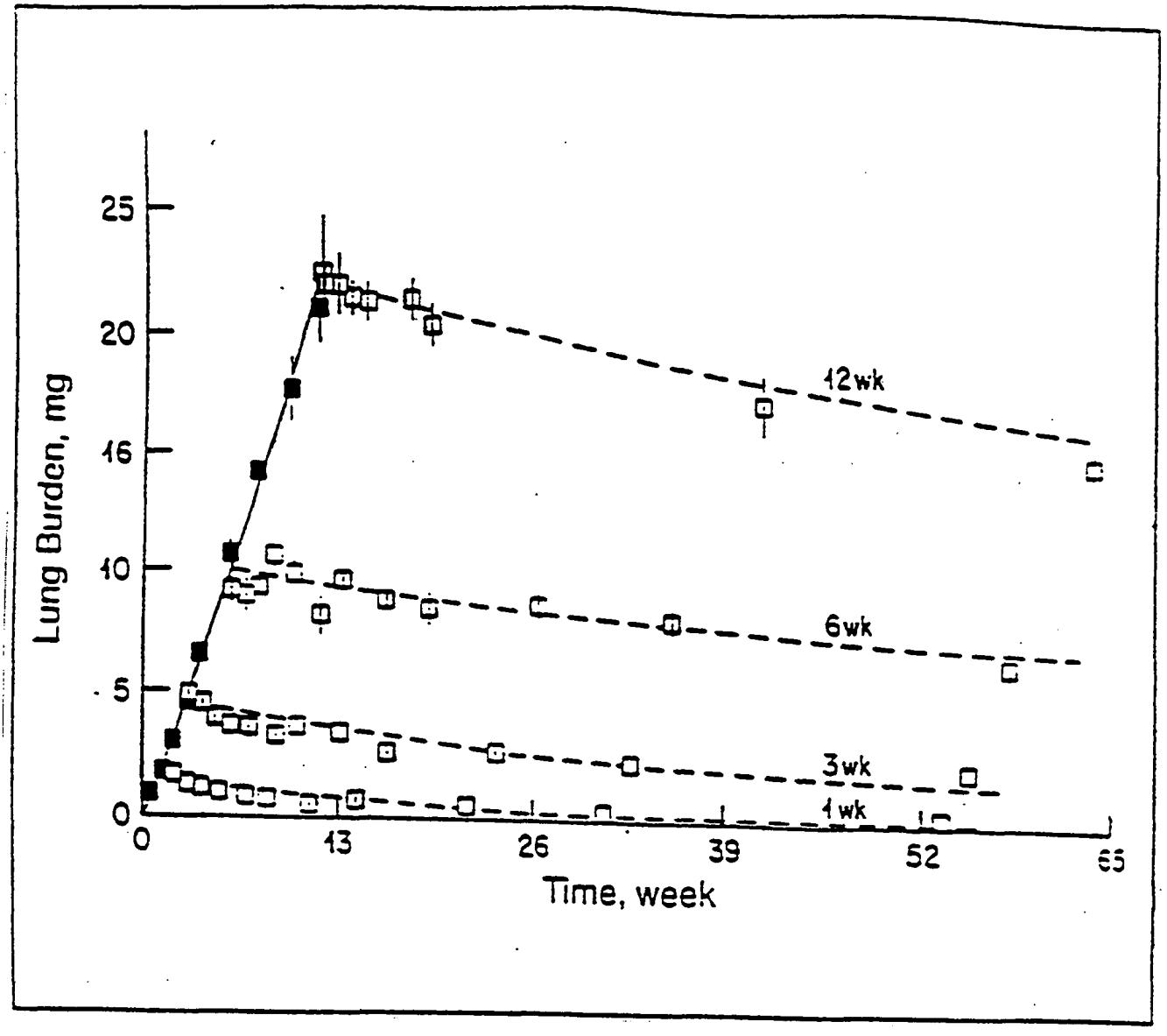

Figure B-3. **The experimental and predicted lung burdens of rats to DEPs at a solid and dashed concentration of 0.6 mg/m^3 for different exposure spans. Lines are, respectively, the predicted burdens during exposure and post-exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).**

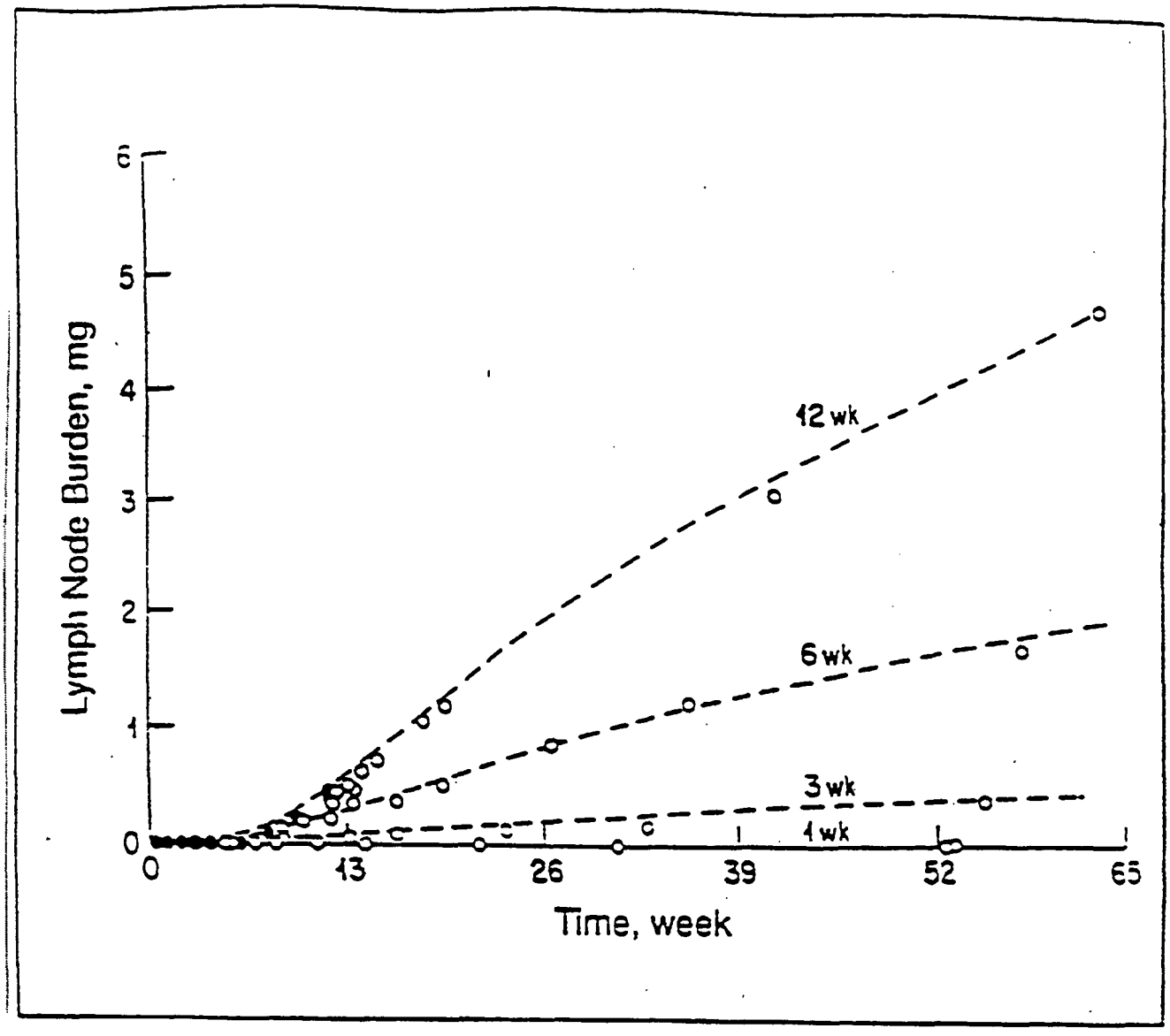

Figure B-4. **Experimental and predicted lymph node burdens of rats exposed to CEPs at a concentration of 6.0 mg/m^3 for different exposure spans. The solid and dashed lines are, respectively, the predicted burdens during exposure and post-exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).**

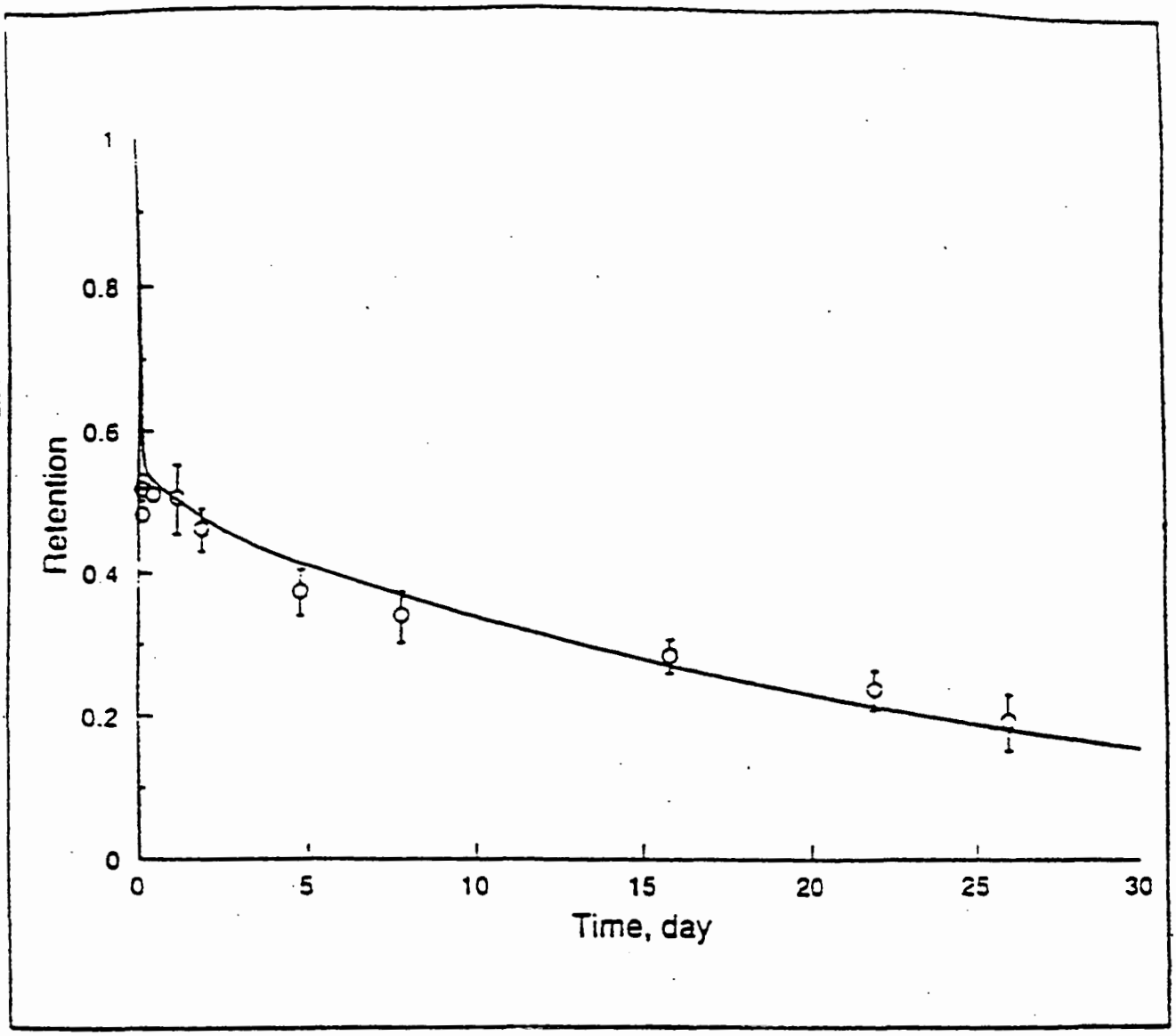

Figure B-5. Comparison between the calculated lung retention (solid line) and the experimental data obtained by Sun et al. (1984) for the particle-associated BaP in rats.

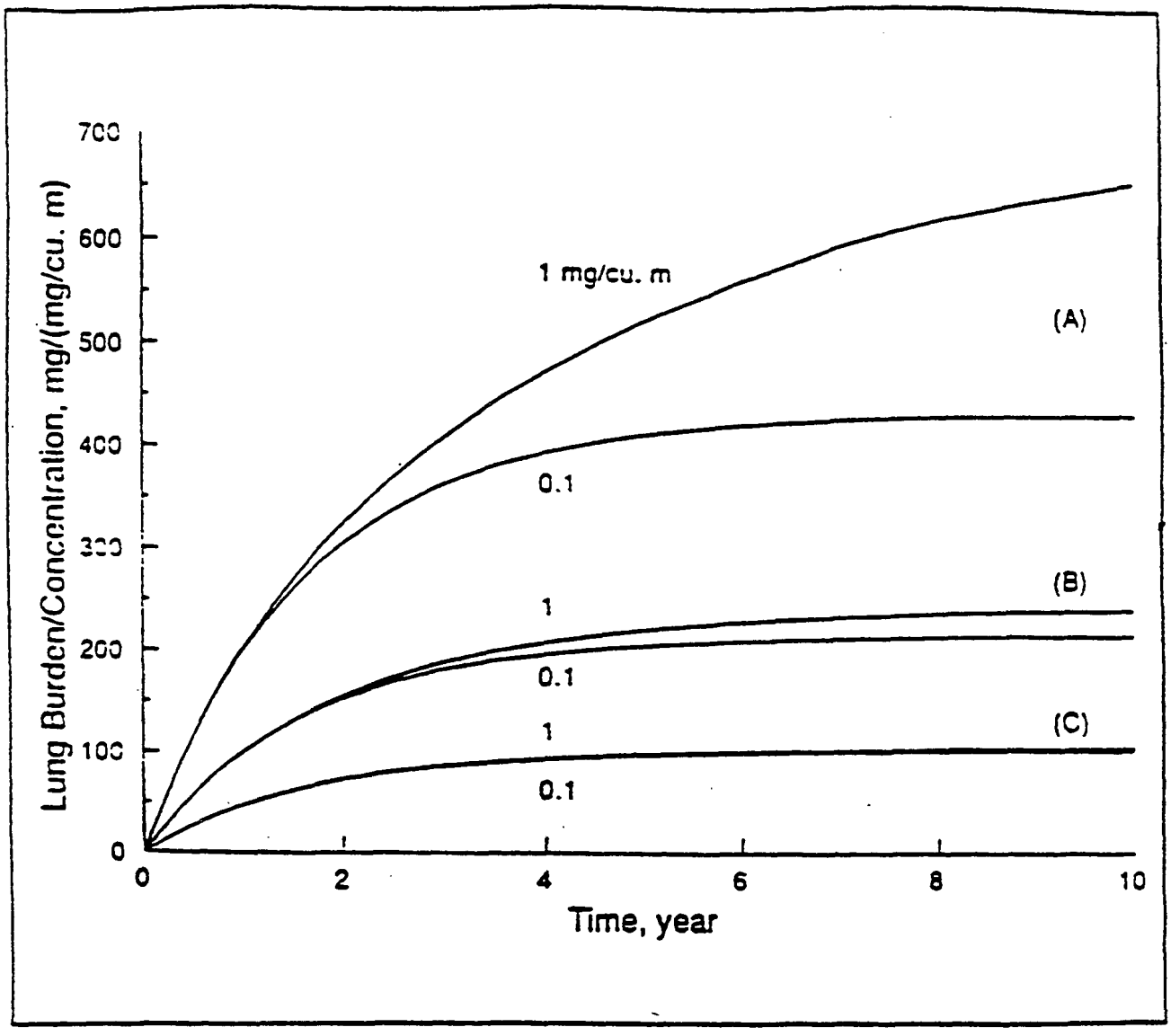

Figure B-6. Calculated lung burdens of diesel soot per unit exposure concentration in human adults exposed continuously to DEPs at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

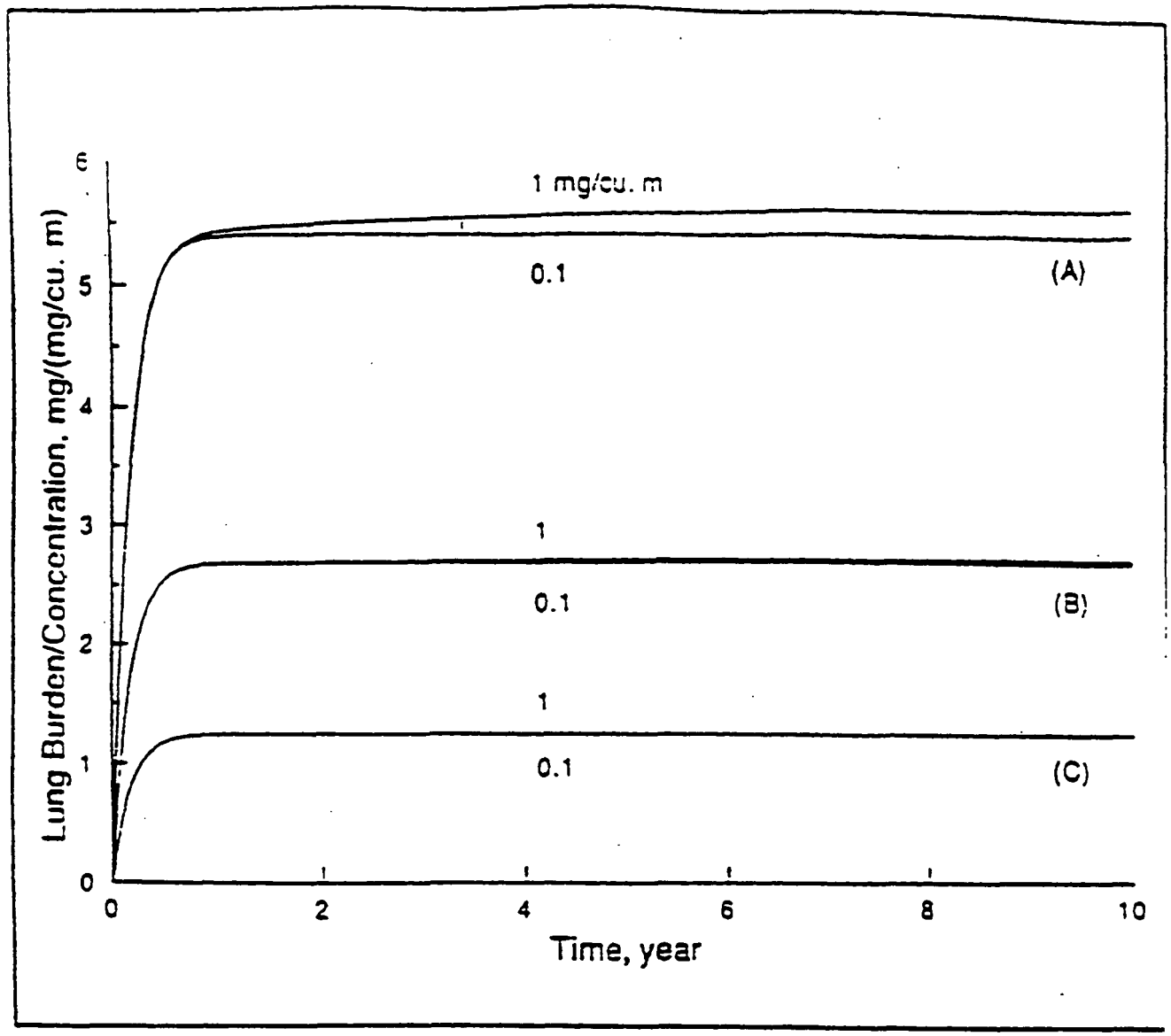

Figure B-7. Calculated lung burdens of the particle-associated organics per unit exposure concentration in human adults exposed continuously to DEPs at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

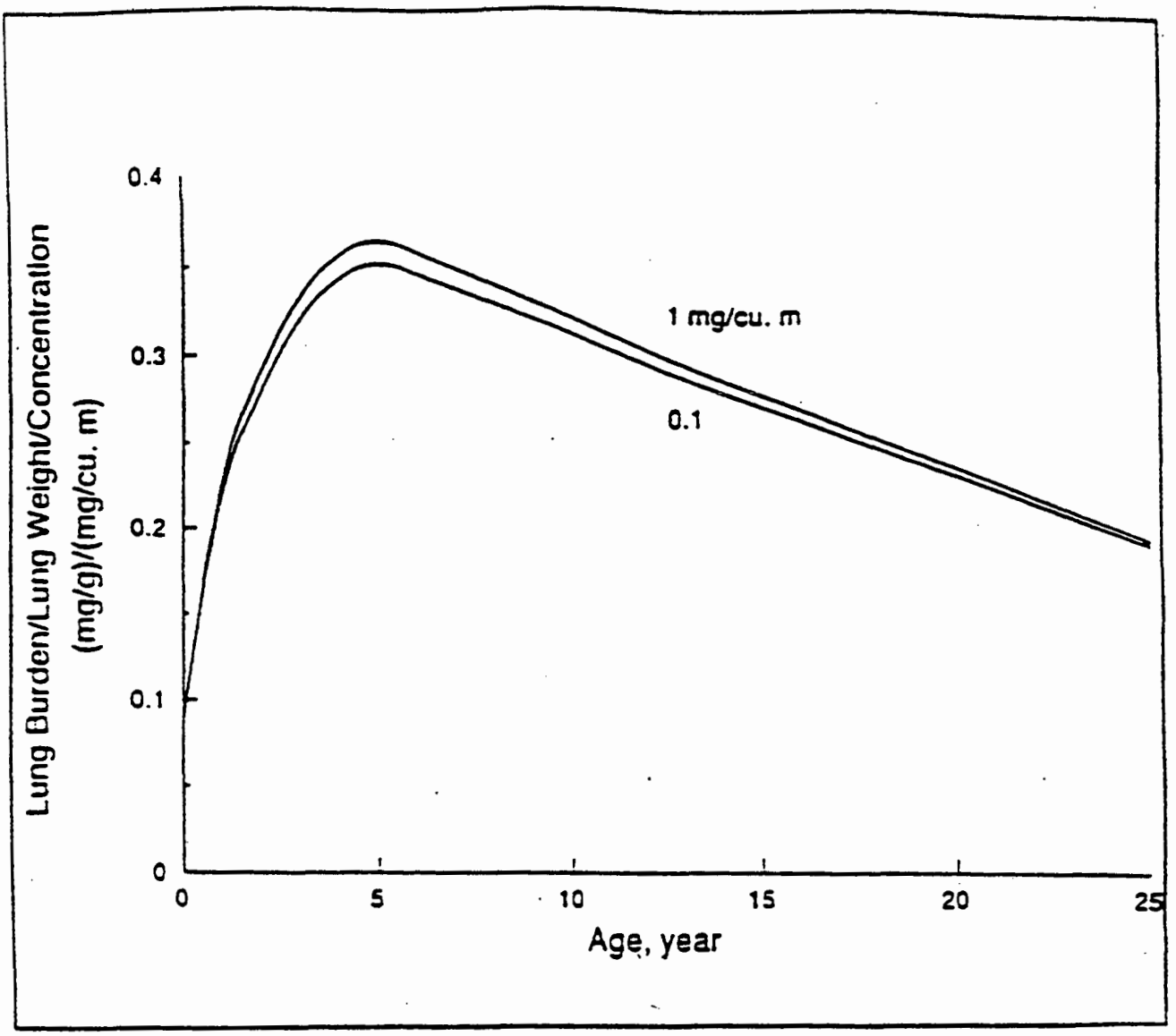

Figure B-8. Calculated lung burdens of diesel soot per gram of lung per unit exposure concentration in humans of different ages exposed continuously for 1 year to DEPs of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.

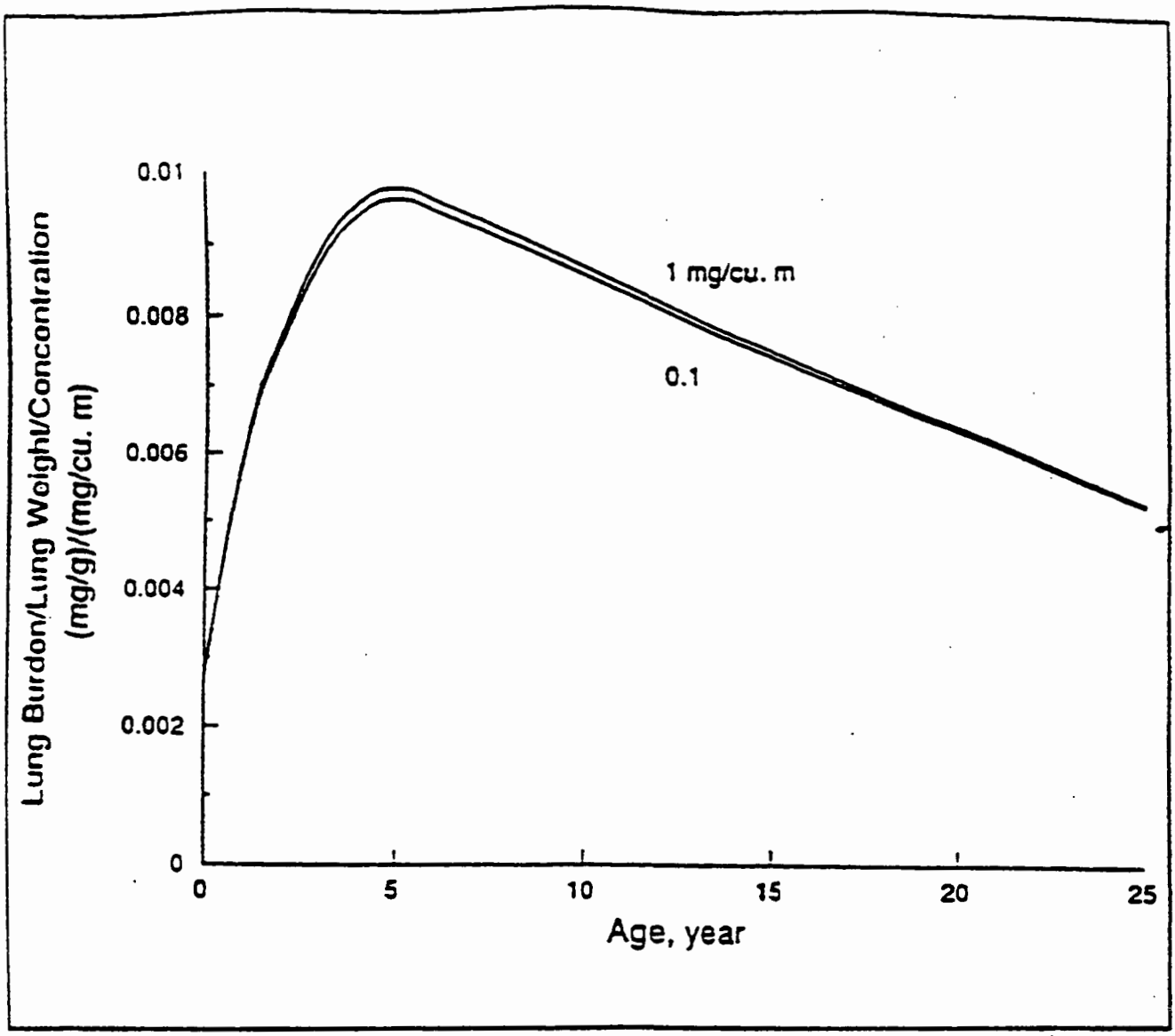

Figure B-9. **Calculated burdens of the particle-associated organics per gram of lung per unit exposure concentration in humans of different ages exposed continuously for 1 year to DEPs of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.**

the amount of particle intake, the steady-state lung burdens per unit concentration were highest for exposure pattern (a) and lowest for exposure pattern (b). Also, increasing soot concentration 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.

Figures B-8 and B-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 1 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 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.

B.8. PARAMETRIC STUDY OF THE MODEL

The deposition and clearance model of DEPs in humans, presented above, consists of a large number of parameters that 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.

B.8.1. Effect of Ventilation Conditions

The changes in lung burden due to variations in tidal volume and respiratory frequency are depicted in Figures B-10 and B-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.

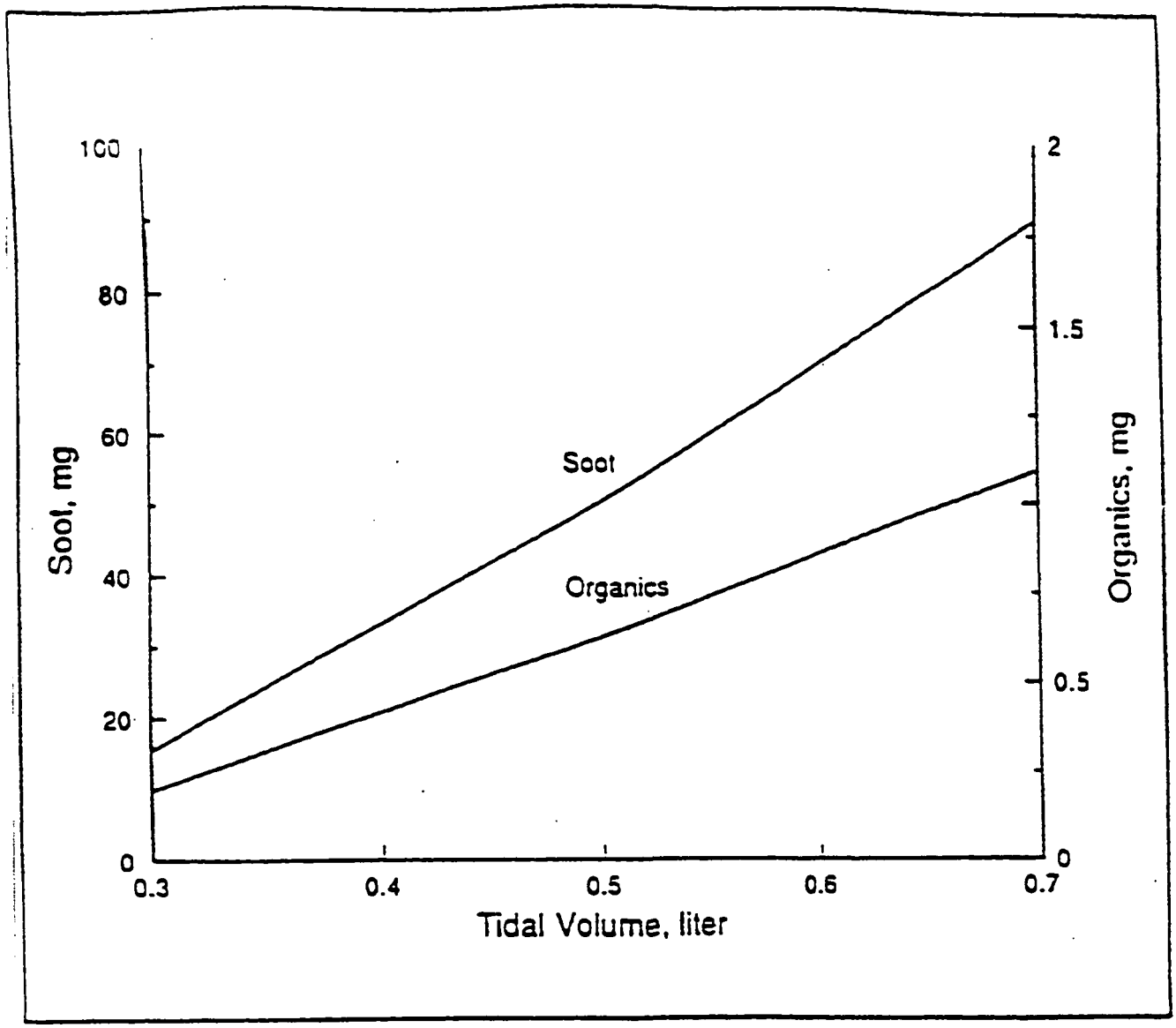

Figure B-10. **Calculated lung burdens in human adults versus tidal volume in liters for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) respiratory frequency = 14 min^{-1}; and (c) lung volume = 3000 cm^3.**

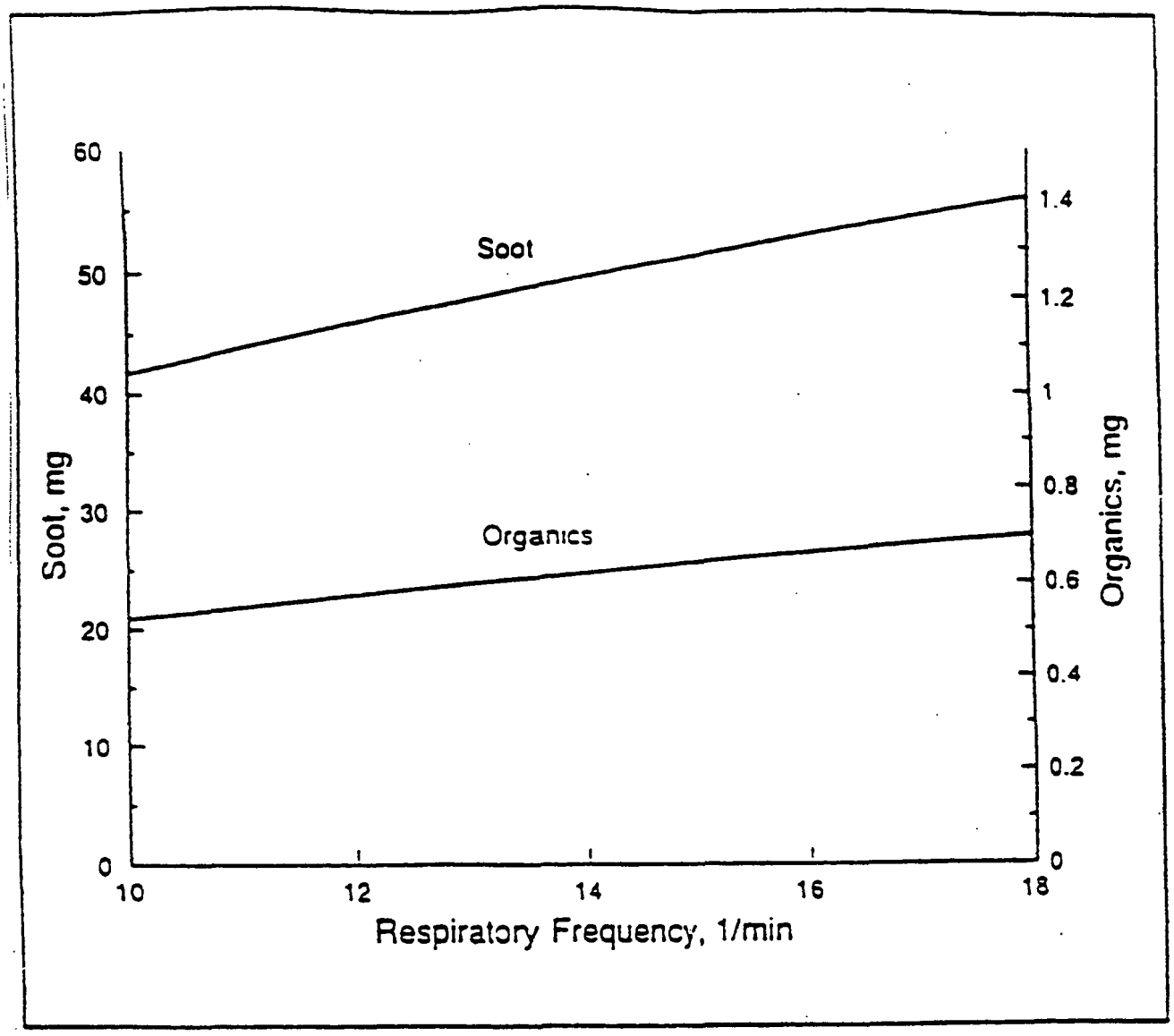

Figure B-11. **Calculated lung burdens in human adults versus respiratory frequency in *bpm* for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, and (c) lung volume = 3200 cm^3.**

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.

B.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. 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 B-12 and B-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 B-12), while just the opposite occurred when the multiple of $\lambda_A^{(2)}$ was increased (Figure B-13).

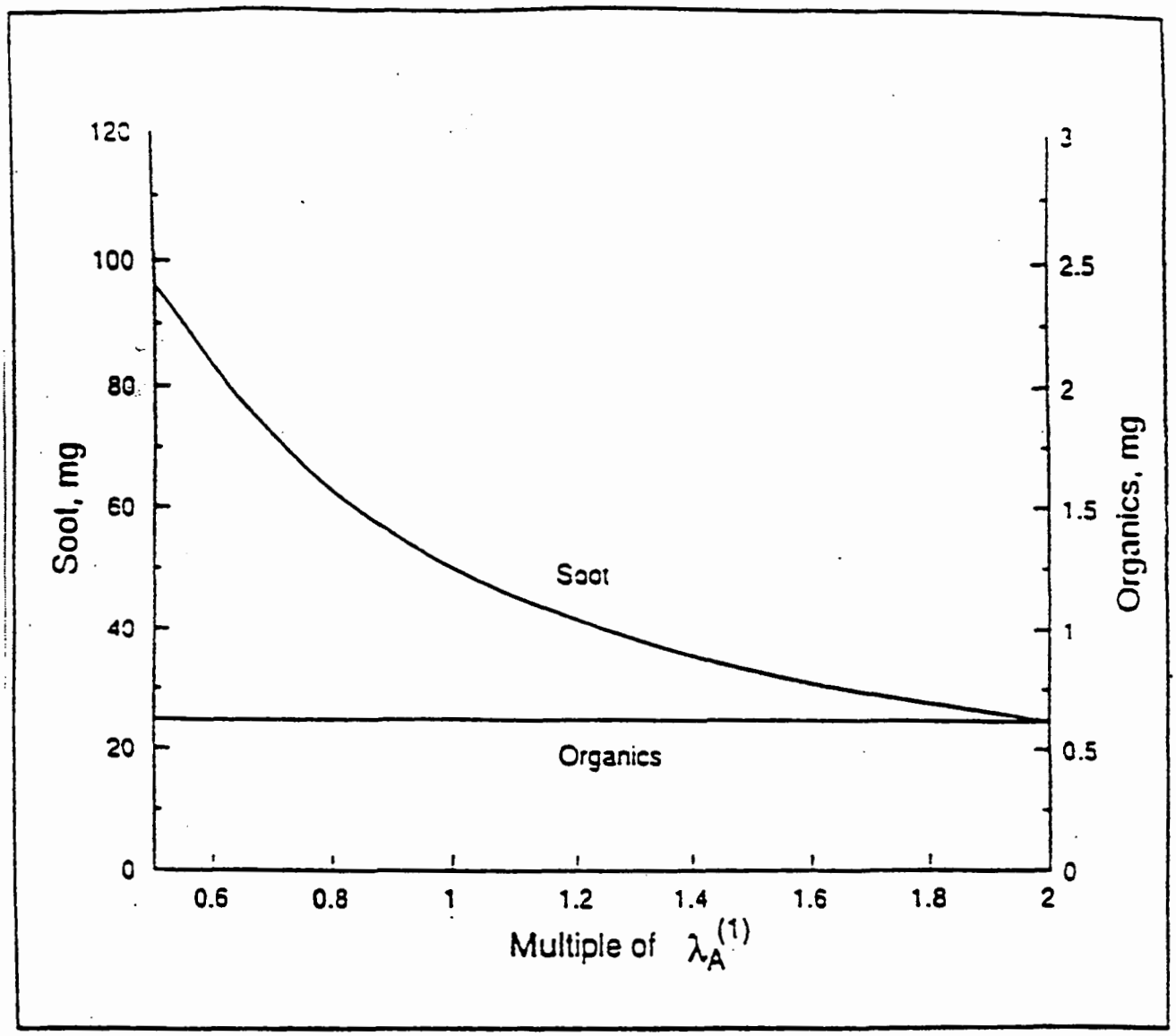

Figure B-12. **Calculated lung burdens in human adults versus multiple of $\lambda_A^{(1)}$ for exposure to DEPs at 0.1 mg/m$_3$ for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}; and (c) lung volume = 3200 cm^3.**

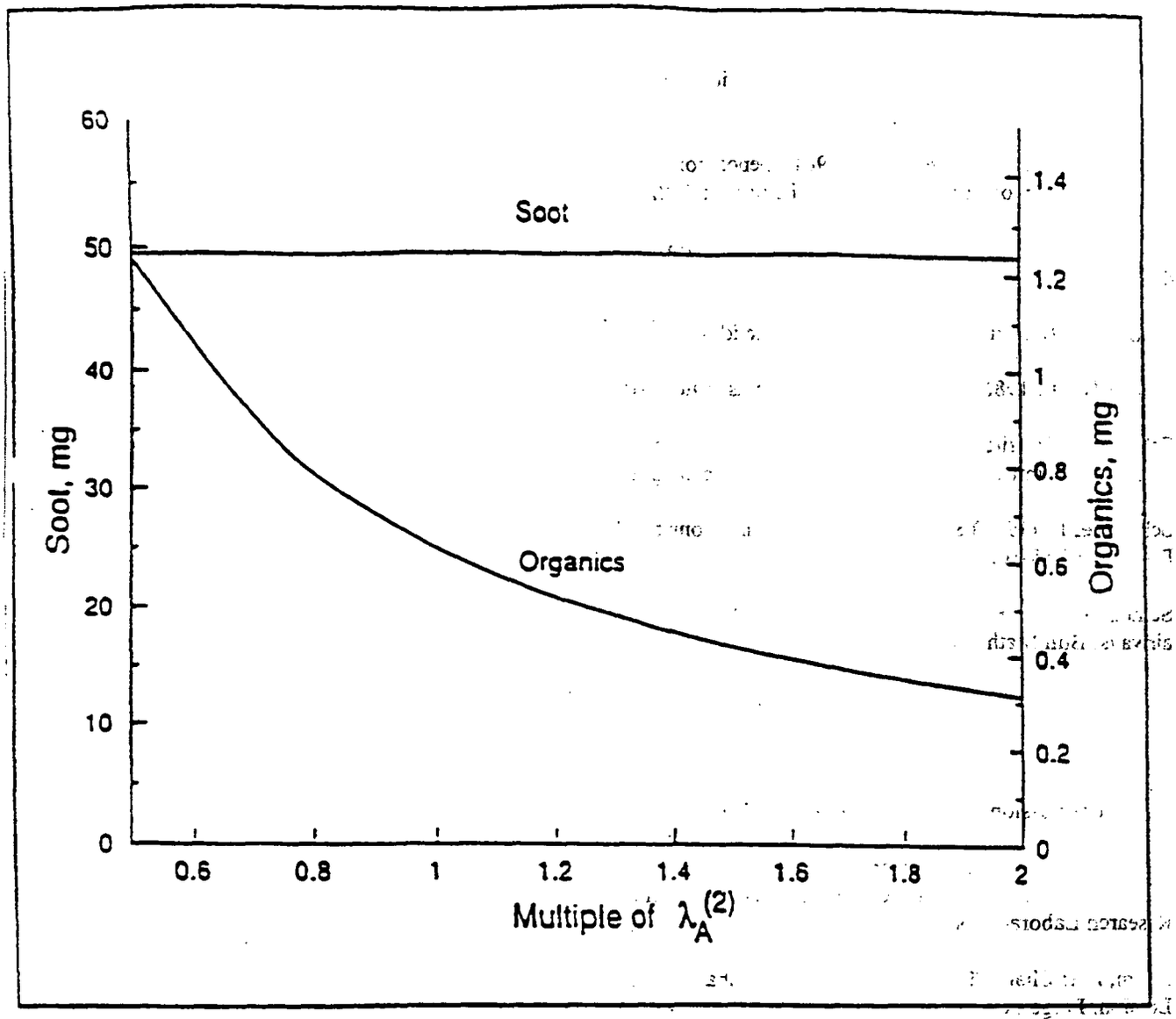

Figure B-13. Calculated lung burdens in human adults versus multiple of $\lambda_A^{(1)}$ for exposure to DEPs at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}; and (c) lung volume = 3200 cm^3.

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