6. ESTIMATING HUMAN NONCANCER HEALTH RISKS OF DIESEL EXHAUST

6.1. INTRODUCTION

As discussed earlier in this document (Chapter 2, Section 2.2.7, 2.2.8), diesel engine exhaust (DE) consists of a complex mixture of gaseous pollutants and particles. In attempting to estimate potential health risks associated with human exposure to DE, researchers have focused attention mostly on the particulate matter (PM) components. They have done so, in part, by comparing the relative toxicity of unfiltered versus filtered DE (with gaseous components removed), as discussed in Chapter 5.

Diesel particulate matter (DPM) consists mainly of: (a) elemental carbon (EC) particles having relatively large surface areas, (b) soluble organic carbon, including 5-ring or higher polycyclic aromatic hydrocarbons (PAHs) such as benzo(*a*)pyrene, and other 3- or 4-ring compounds distributed between gas and particle phases, and (c) metallic compounds. DPM also typically contains small amounts of sulfate/sulfuric acid and nitrates, trace elements, and water, plus some unidentified components. DPM is made up almost entirely of fine particles (i.e., all below 1–3 μm) with a significant subset of ultrafine particles (i.e., those with a mass median diameter below about 0.1 μm).

Health concerns have long focused on DPM. Toxicological data described in Chapter 5 (Section 5.2) indicate DPM to be the prime etiologic agent of noncancer health effects when DE is sufficiently diluted to limit the concentrations of gaseous irritants (NO_2 and SO_2), irritant vapors (aldehydes), CO, or other systemic toxicants. The large surface areas of DPM allow for adsorption of organics from the diesel combustion process and for adsorption of additional compounds during transport in ambient air. The small size of DPM, combined with their large surface area, likely enhance the potential for subcellular interactions with important cellular components of respiratory tissues once the particles are inhaled by humans or other species (Johnston et al., 2000; Oberdörster et al., 2000).

The content of DPM as described above and in Chapter 2 is of clear toxicological significance. The experimental evidence described in Chapter 5 concerning DPM's association with and etiology of noncancer effects is extensive and compelling. These points, along with the fact that DPM is easily and most frequently measured and reported in toxicological studies of diesel emissions, make DPM a reasonable choice as a measure of diesel emissions. As a surrogate, DPM is as valid as any other component of DE to show what is currently known—and probably what is not yet known—about diesel emissions. Therefore, DPM is the quantitative focus of this chapter.

The usual agency approach to evaluating noncancer risks from inhaled exposures to toxic air pollutants such as ambient DE has been documented by EPA in the methods for derivation of an inhalation reference concentration (RfC) (U.S. EPA, 1994). For DPM exposures, this means combining key elements derived from evaluations of specific DPM noncancer effects in animals and humans (described in Chapter 5) with the use of quantitative dosimetry models (described in Chapter 3). The goal is to estimate DPM concentrations to which humans might be exposed throughout their lives (i.e., chronically) without experiencing any untoward or adverse effects. Such an effort can be accomplished through analysis of dose-response relationships where the adverse response is considered as a function of a corresponding measure of dose. Chapter 5 is replete with dose-response information on adverse (but nonlethal) noncancer health effects observed in long-term (chronic/lifetime) exposure studies to DE in general and to DPM in particular, albeit mostly in animals. Chapter 3 analyzes available methods to convert external exposure concentrations of DPM in animal studies to estimates of a human-equivalent concentration (HEC). The following sections of this chapter (Sections 6.2, 6.3, and 6.5) assess and integrate this information to derive a chronic RfC, using the above-cited methodology in developing dose-response assessments of the noncancer effects of toxic air pollutants.

Yet another approach to consider in deriving quantitative estimates of potential human health risks associated with ambient (nonoccupational) DPM exposures is the extent to which DPM could contribute to the adverse health effects that have been associated with exposure to ambient fine PM, $PM_{2.5}$. Such associations with adverse health effects are based primarily on epidemiologic studies evaluated in EPA's Air Quality Criteria Document for Particulate Matter (PM CD) (U.S. EPA, 1996a).[1] This PM CD served as the scientific basis for the last periodic review of the national ambient air quality standards (NAAQS) for PM, which resulted in the establishment of revised PM standards in 1997, including standards for $PM_{2.5}$. DPM is a component of ambient fine PM (see Chapter 2) and should be considered as a toxicologically important component of ambient fine PM. Any guidelines established for DPM, then, should be concordant with information on fine PM in general, as presented in the PM CD. To more fully consider the implications of the relationship between ambient DPM and fine PM, the epidemiological evidence on fine PM and the basis for the $PM_{2.5}$ standards are summarized, and the relationship between ambient DPM and fine PM is discussed later in this chapter (Section 6.4). This relationship is of interest with respect to the noncancer assessment of DE. As is noted here , however, and reflected in Sections 6.2–6.4 below, the definitions, procedures, and statutory mandates that apply to criteria pollutants such as PM (regulated through the

[1]A new PM CD is now being prepared to reflect the latest scientific studies on ambient PM available since the last document was completed.

establishment of NAAQS under sections 108 and 109 of the Clean Air Act) are fundamentally different from those that apply to toxic air pollutants such as DE and to the derivation of RfCs for such pollutants. Thus, the ambient $PM_{2.5}$ concentrations that are specified as the levels of the $PM_{2.5}$ NAAQS should not be compared directly with any RfC that may be derived for DPM. It is reasonable to observe, however, that the annual $PM_{2.5}$ standard would be expected to provide a measure of protection from DPM, reflecting DPM's current approximate proportion to $PM_{2.5}$.

Estimates of DE levels associated with effects occurring under less than lifetime exposure scenarios (such as acute exposure) are not addressed in this chapter. Studies of acute exposure to DE are discussed in Chapter 5, but are accompanied by scant dose-response information, with single-exposure studies for various specialized endpoints (e.g., allergenicity/adjuvancy) and other multiple-exposure-level studies reporting data on mortality only. Based on currently available methodologies, these studies do not yet appear to provide a sufficient basis from which to derive a dose-response assessment for an acute DE exposure scenario.

6.2. THE INHALATION REFERENCE CONCENTRATION APPROACH

Historically, approaches such as the Acceptable Daily Intake (ADI) were developed whereby effect levels, such as no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) from human or animal data, were combined with certain "safety factors" to accommodate areas of uncertainty to make quantitative estimates of a safe dose, i.e., a level at which no adverse effect would be likely to occur. In response to the National Academy of Sciences (NAS) report entitled "Risk Assessment in the Federal Government: Managing the Process" (National Research Council, 1983), EPA developed two approaches similar to the ADI, i.e., the oral reference dose (RfD) (Barnes and Dourson, 1988) and the parallel inhalation reference concentration, the RfC, with its formal methodology (U.S. EPA, 1994). Similar to the ADI in intent, the RfD/C approach is used for dose-response assessment of noncancer effects, using an explicitly delineated, rigorous methodology that adheres to the principles set forth in the 1983 NRC report. The RfC methodology includes comprehensive guidance on a number of complex issues, including consistent application to effect levels of uncertainty factors (UFs) rather than the ADI safety factors for consideration of uncertainty. Basically, these approaches attempt to estimate a likely subthreshold concentration in the human population. Use of the RfD/C approach is one of the principal current agency methods for deriving dose-response assessments.

A chronic RfC is currently defined as:

An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime.

The RfC approach involves the following general steps:

- Identification of a critical effect relevant to humans, i.e., an adverse effect that occurs at the lowest exposure/dose in human or animal studies and whose prevention avoids the occurrence of all other adverse effects;
- Selection of appropriate dose-response data to derive a point of departure (POD) for extrapolation of a key study (or studies) that provides a NOAEL, LOAEL, or benchmark concentration ($BMCL_x$)[2];
- Estimation of HECs when animal exposure-response data are used (via use of PBPK/dosimetry models);
- Application of UFs to the point of departure (e.g., NOAEL, LOAEL, $BMCL_x$) to address extrapolation uncertainties (e.g., interindividual variability, interspecies differences, adequacy of database); and
- Characterization of the "confidence" in the dose-response assessment and resultant RfC.

The basic quantitative formula for derivation of an RfC, given in Equation 6-1, has as its basic components an effect level, here a NOAEL, expressed as an HEC, and UFs. The units of an RfC are typically mg/m^3 or $\mu g/m^3$.

Alternatively, the numerator in Equation 6-1 may be a LOAEL or $BMCL_x$. The

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

benchmark concentration (BMC) approach and its application in this assessment are documented in Appendix B and described further below. Also, a modifying factor (MF) may be used in the denominator of this equation to account for scientific uncertainties, usually relating to the study chosen as the basis for the RfC. Further specifics of RfC derivation procedures are discussed as they are used in the following sections. All such procedures are described in detail in the RfC Methodology (U.S. EPA, 1994).

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[2]$BMCL_x$ is defined as the lower 95% confidence limit of the dose that will result in a level of "x" response (e.g., $BMCL_{10}$ is the lower 95% confidence limit of a dose for a 10% increase in a particular response). See Appendix B for further specifics.

6.3. CHRONIC REFERENCE CONCENTRATION FOR DIESEL EXHAUST

As concluded in Chapter 5, chronic respiratory effects are the principal noncancer hazard to humans from long-term environmental exposure to DE. Other effects (e.g., neurological, liver-related) are observed in animal studies at higher exposures than those producing the respiratory effects. The human and animal data for the immunological effects of DE are currently considered inadequate for dose-response evaluation. Thus, the respiratory effects are considered the "critical effect" for the derivation of a chronic RfC for DE.

The evidence for chronic respiratory effects is based mainly on animal studies showing consistent findings of inflammatory, histopathological (including fibrosis), and functional changes in the pulmonary and tracheobronchial regions of laboratory animals, including the rat, mouse, hamster, guinea pig, and monkey. Occupational studies of DE provide some corroborative evidence of possible respiratory effects (e.g., respiratory symptoms and possible lung function changes), although those studies are generally deficient in exposure information.

Mode-of-action information about respiratory effects from DE exposure indicates that, at least in rats, the pathogenic sequence following the inhalation of DPM 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. This series of events may result in pulmonary inflammation, fibrosis, and eventually lesions like those described in the studies reviewed in Chapter 5. Although information describing the possible pathogenesis of respiratory effects in humans is not available, the effects reported in studies of humans exposed to DE are not inconsistent with the findings in controlled laboratory animal studies.

Several reasons explain why the dose-response data from rats are considered especially appropriate for use in characterizing noncancer health effects in humans and deriving a chronic RfC for DE. First, similar noncancer respiratory effects are seen in other species (mouse, hamster, guinea pig, and monkey). Second, rats and humans exhibit similar noncancer responses (macrophage response and interstitial fibrosis) to other particles such as coal mine dust, silica, and beryllium (Haschek and Witschi, 1991; Oberdörster, 1994). Third, relative to other species there exists a plethora of long-term, specialized, and mechanistic studies in rats. Fourth, an expert panel convened by the International Life Sciences Institute (ILSI) recommended that

response data on persistent, inflammatory processes may be used to assess nonneoplastic responses of poorly soluble particles (PSP) such as DPM (ILSI, 2000).

6.3.1. Principal Studies for Dose-Response Analysis: Chronic, Multiple-Dose Level Rat Studies

The experimental protocols and results from the long-term, repeated-exposure chronic studies demonstrating and characterizing the critical effects of pulmonary fibrotic changes and inflammation are discussed in Chapter 5. Salient points of these studies, including species/sex of the test species, the exposure regime and concentrations reported in mg DPM/m^3, and effect levels, are abstracted in Table 6-1 for further consideration. The effect levels are designated as N for no-observed-adverse-effect level, A for adverse-effect level, and $BMCL_{10}$.

The purpose of many of the chronic studies listed in this table was not the elucidation of the concentration-response character of DPM. The studies of Heinrich et al. (1982, 1986) in hamsters, mice, and rats; of Iwai et al. (1986) in rats; of Lewis et al. (1989) in monkeys; and of Pepelko (1982a) in rats are all single-dose-level analyses that have as their genesis mechanistic or species-comparative purposes. As discussed in Chapter 5, many of these studies do provide valuable supporting information for designation of the critical effect of pulmonary histopathology. The lack of any clear dose-response data, however, precludes consideration of these studies as a basis for RfC derivation.

Likewise, studies of chronic, multiple-level exposure involving species other than rats, i.e., hamsters (Pepelko, 1982b), cats (Plopper et al., 1983), and guinea pigs (Barnhart et al., 1981, 1982), provide cross-species corroboration of the critical effects of pulmonary histopathology and inflammatory alteration.

The remaining studies showing exposure-response relationships in rats for the critical effects include those of Ishinishi et al. (1986, 1988), Mauderly et al. (1987a), Heinrich et al. (1995), and Nikula et al. (1995). As described in Chapter 5, all of these studies were conducted and reported in a thorough, exhaustive manner on the critical effects and little, if any, basis exists for choosing one over another for purposes of RfC derivation. One way of taking advantage of this high degree of methodological and scientific merit would be to array data from all these studies and their effect levels (NOAEL, LOAEL, $BMCL_x$) subsequent to normalization of the exposure conditions, i.e., conversion of the exposure regimes to yield an HEC. This exercise would result in an interstudy concentration-response continuum normalized to a continuous human exposure to DPM that would facilitate the choice of a concentration to use as a point of departure in deriving an RfC.

Table 6-1. Histopathological effects of diesel exhaust in the lungs of laboratory animals

Study	Species/sex	Exposure period	Particles (mg/m^3)	Effect level[a]	Effects[b]
Lewis et al. (1989)	Monkey, Cynomolgus, M	7 h/day 5 days/wk 104 wks	2.0	N	AM aggregation; no fibrosis, inflammation, or emphysema
Bhatnagar et al. (1980) Pepelko (1982a)	Rat, F344, M, F	7 h/day 5 days/wk 104 wks	2.0		Multifocal histiocytosis; inflammatory changes; Type II cell proliferation; fibrosis
Pepelko (1982b)	Hamster, Chinese, M	8 h/day 5 days/wk 26 wks	6.0 12.0	A	Inflammatory changes; AM accumulation; thickened alveolar lining; Type II cell hyperplasia; edema; increase in collagen
Heinrich et al. (1982)	Hamster, Syrian, M, F	7-8 h/day 5 days/wk 120 wks	3.9	A	Inflammatory changes, 60% adenomatous cell proliferation
Iwai et al. (1986)	Rat, F344, F	8 h/day 7 days/wk 104 wks	4.9	A	Type II cell proliferation; inflammatory changes; bronchial hyperplasia; fibrosis
Mauderly et al. (1987a) Henderson et al. (1988)	Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/wk 130 wks	0.35 3.5 7.1	N A A	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; few quantitative data given
Heinrich et al. (1995)	Rat, Wistar, F; Mouse, NMRI, F (7 mg/m^3 only)	18 h/day 5 days/wk 24 mo	0.8 2.5 7.0	A A A	Bronchioalveolar hyperplasia, interstitial fibrosis in all groups; severity and incidence increase with exposure concentration; text given only
	Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/wk 13.5 mo (NMRI) 24 mo (C57BL/N)	7.0	A	No increase in tumors; noncancer effects not discussed

Table 6-1. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)

Study	Species/sex	Exposure period	Particles (mg/m^3)	Effect level[a]	Effects[b]
Ishnishi et al. (1986, 1988)	Rat, M, F, F344, /Jcl.	16 h/day 6 days/wk 130 wks	0.11[c] 0.41[c] 1.08[c] 2.32[c] 0.46[d] 0.96[d] 1.84[d] 3.72[d]	N N A A N A A A	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; data given in text only
Heinrich et al. (1986)	Hamster, Syrian, M, F; Mouse, NMRI, F; Rat, Wistar, F	19 h/day 5 days/wk 120 wks	4.24	A	Inflammatory changes; thickened alveolar septa; bronchioloalveolar hyperplasia; alveolar lipoproteinosis; emphysema (diagnostic methodology not described); hyperplasia; lung tumors
Barnhart et al. (1981, 1982); Vostal et al. (1981)	Guinea pig, Hartley, M	20 h/day 5.5 days/wk 104 wks	0.25 0.75 1.5 6.0	N A A A	Minimal response at 0.25 and 0.75 mg/m^3; 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
Plopper et al. (1983) Hyde et al. (1985)	Cat, inbred, M	8 h/day 7 days/wk 124 wks	6.0[c] 12.0[d]	A A	Inflammatory changes; AM aggregation; bronchiolar epithelial metaplasia; Type II cell hyperplasia; peribronchiolar fibrosis
Nikula et al. (1995)	Rat, F344, M	16 h/day 5 days/wk 23 mo	2.44 6.33	A, A $BMCL_{10}$	AM hyperplasia, epithelial hyperplasia, inflammation, septal fibrosis, bronchoalveolar metaplasia

[a]N= no-observed-adverse-effect level; A = adverse-effect level; $BMCL_{10}$ = benchmark concentration, lower limit, at a 10% response level (for incidence); see Appendix A for further specifics.
[b]AM = Alveolar macrophage; PMN = Polymorphonuclear leukocyte
[c]Light-duty engine.
[d]Heavy-duty engine.
[e]1 to 61 weeks exposure.
[d]62 to 124 weeks of exposure.
[e]See Appendix A.

6.3.2. Derivation of Human Continuous Equivalent Concentrations, HECs

Pharmacokinetic, or PK, models can be used to estimate across species the external concentrations of a toxicant that would result in equivalent internal doses. When used for these purposes, PK models may be termed comparative dosimetric models. Chapter 3 reviewed and evaluated a number of dosimetric models applicable to DPM. This analysis indicated that outputs from the human component of the model developed by Yu et al. (1991) specifically for DPM, such as deposition and estimated lung burden, were not substantially different from other available models. The analysis also demonstrated that the Yu model accounted for several diesel-specific phenomena, including particle overload lung clearance rates and interspecies kinetics of desorption of organics from the carbonaceous core of DPM, both slow- and fast-cleared. Of importance, the Yu model was parameterized for deposition and clearance in both animals and humans. Also, the animal component of the model was based on data from rats actually exposed to DPM, whereas other models analyzed used data based only on generic particles in the size range of DPM. It was concluded from this analysis that the Yu model could be used to estimate disposition of DPM both in animals and in humans and would therefore be an acceptable choice in performing animal-to-human extrapolation in deriving a continuous human-equivalent concentration. Note, however, that use of this or any other available PK model would address species differences in dose (i.e., pharmacokinetics, PK), and not necessarily pharmacodynamics (PD), the other component of uncertainty in animal-to-human or interspecies extrapolation (U.S. EPA, 1994).

Guidance on choosing measures of exposure for poorly soluble particles such as DPM (ILSI, 2000) states that some measures of external dose (e.g., the aerosol exposure parameters of MMAD, σ_g, particle surface area, and density) should be characterized. Likewise, some indication of internal dose resulting from the external exposure (e.g., lung burden) should be measured so that differences in dose metrics may be considered as new mechanistic insights are developed. The whole particle, as characterized in this assessment and used in the model of Yu et al. (1991), meets this recommended guidance, and DPM, in $\mu g/m^3$, is used as the measure of external exposure. Internal measures of exposure or dose were also considered in Chapter 3 (Section 3.3.1.1) with the conclusion that the dose metric of lung burden of DPM in terms of surface area (mg/cm^2) at the termination of the exposure period appears to be the most defensible and appropriate measure of internal dose, especially where clearance is involved. More detailed specifics are available in Chapter 3 and in Appendix A.

The logical and operational sequence of deriving a HEC using the Yu model and these metrics, i.e. external air concentration (in $\mu g/m^3$) and lung burden (in mg/cm^2), is demonstrated in Figure 6-1. First, the experimental animal exposures, including external concentration and

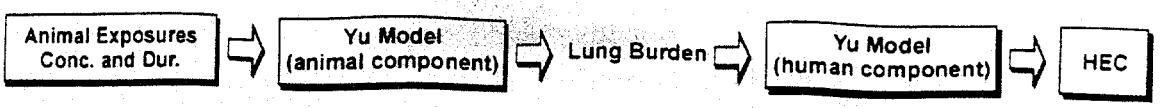

Figure 6-1. Flow diagram of procedure for calculating HECs.

daily and weekly duration, are entered into the animal component of the Yu model to estimate the animal lung burden, in mg DPM/cm^2, for the specific exposure scenario. The human component of the Yu model is then used by setting desired exposure conditions (continuous for 70 years) and running the model to find an external exposure DPM concentration that would result in this same lung burden. The human external DPM concentration matching this lung burden is the human-equivalent concentration. The step-by-step specifics and results of this procedure as applied to the various studies in Table 6-1 are shown in Table A-4 and fully explained in Appendix A.

The foregoing discussion does not address the variability in outcomes that may be estimated from the Yu et al. (1991) model from deposition of DPM. The model comparison exercises in Chapter 3 showed relatively minor differences among the various human models for one measure, deposition, and indicated that human lung burdens estimated by the human component of the Yu and ICRP66 models were nearly identical at low-exposure concentrations. Variability in output of their model (lung burden) was also examined by Yu and Yoon (1990), who studied dependency on tidal volume, respiration rate, and clearance (in terms of the overall particle transport rate from the alveolar region, λ_A). Analysis indicated that the model output is sensitive, but not overly so, for these determinative parameters. A ± 20% change in values for λ_A, for example, was estimated to result in a 16%–26% change in soot burden at a 0.1 mg/m^3 continuous diesel exposure for 10 years. For a ± 10% change in tidal volume, the model projected changes in soot burden ranging from 14% to 22% for this same exposure scenario. The fact that the changes in the model outcome were comparable to changes in the input parameters, such as tidal volume, indicates that the variability of the model when applied to the human population would reflect the variability of these physiological parameters across that population. In sum, at low concentrations of DPM (< 0.5 mg/m^3), relatively minor differences exist among the models currently available, and the input parameters in the human population may be a major source of variability. As discussed below, variability within the human population often is addressed by applying safety or uncertainty factors, usually in the range of 10 (Renwick and Lazarus, 1998; U.S. EPA, 1994).

6.3.3. Dose-Response Analysis—Choice of an Effect Level

HECs were obtained for the dose levels and exposure scenarios presented in the studies of Mauderly et al. (1987b), Ishinishi et al. (1986, 1988), Nikula et al. (1995), and Heinrich et al. (1995), the specifics of which are presented in Appendix A, specifically Table A-4. The HECs, along with the corresponding specific lung burdens in terms of $\mu g/cm^2$, were transcribed from Table A-4 and, along with the accompanying effect level (NOAEL, LOAEL or $BMCL_{10}$), are arrayed ordinally in Table 6-2. It is acknowledged that Table 6-2 is by no means a full portrayal of the dose-response relationship that may exist for DPM and health effects.

As indicated by the $BMCL_{10}$ values listed for the Nikula et al. (1995) study in Table 6-2, the BMC analysis was carried out on the DPM database and is documented in Appendix B. The chronic rat studies identified in this chapter were analyzed for information suitable for BMC analysis. Results yielded only a few datasets of pulmonary toxicity data from a single study, that of Nikula et al. (1995), that could be used for BMC analysis. These pulmonary data (histopathology incidence data) were extracted, HEC concentrations were calculated using the model of Yu, and the BMCs were generated. The results yielded a complex array of $BMCL_{10}$s from three different effects in two sexes (both separate and combined) with nine different models that were evaluated based on the nature of the dataset, on the goodness-of-fit parameters, and on visual inspection of the graphical outputs. From among all the benchmark data generated, the $BMCL_{10}$ of 0.37 mg/m^3 calculated from combined male and female rat pulmonary histopathology was judged as the most defensible choice. However, further characterization of this same benchmark value indicates that it is not a suitable candidate for use as a point of departure for development of a dose-response assessment such as the RfC. Limitations included the excessive extent of extrapolation from the observed experimental range (see Figure B-1 in Appendix B) and the paucity of data points (there were only two exposure groups) overall. Another serious limitation is that the high experimental concentrations used (and their $C \times t$ product) are well in the range where the problematic phenomenon of pulmonary overload in rats occurs (Section 5.1.3.3.4).

Inspection of Table 6-2 shows that calculating and ordering the HECs created a partial concentration-response continuum reflected in the estimated internal lung burden also given in this table. The continuum extends from HECs with no observed adverse effects at concentrations as low as 0.032 mg/m^3 to as high as 0.144 mg/m^3 to HECs with an adverse effect level that first appears definitively in the continuum probably at 0.33 mg/m^3 and extends out to 1.95 mg/m^3.

It should be noted that the relationship between HEC and lung burden is not consistently proportional. For example, at the lowest HEC listed, 0.032 mg/m^3, a lifetime (70 years) of continuous exposure to this concentration is estimated to result in a specific burden to the lung of 0.0587 $\mu g/cm^2$. At the other end of this spectrum, a lifetime of continuous exposure to 4.4

Table 6-2. Human equivalent continuous concentrations: 70-year HECs calculated with the model of Yu et al. (1991) from long-term studies of rats repeatedly exposed to DPM[a]

Study	Exposure concentration (mg/m^3)	Effect level[a]	Lung burden (modeled) (μg DPM $/cm^2$)[b]	HEC (mg/m^3)
Ishinishi et al. (1988) (LD[c])	0.11	NOAEL	0.0587	0.032
Mauderly et al. (1987a)	0.35	NOAEL	0.0685	0.038
Ishinishi et al. (1988) (LD)	0.41	NOAEL	0.245	0.128
Ishinishi et al. (1988) (HD)	0.46	NOAEL	0.281	0.144
Heinrich et al. (1995)	0.84	LOAEL	0.94	0.33
Nikula et al. (1995)	2.44 & 6.3[d]	$BMCL_{10}$ -inflam	1.34	0.37
Ishinishi et al. (1988) (HD)	0.96	LOAEL	3.16	0.883
Ishinishi et al. (1988) (LD)	1.18	LOAEL	4.50	1.25
Nikula et al. (1995)	2.44 & 6.3[d]	$BMCL_{10}$ - fibrosis	4.70	1.3
Mauderly et al. (1987a)	3.47	LOAEL	4.95	1.375
Nikula et al. (1995)	2.44	LOAEL	7.00	1.95
Ishinishi et al. (1988) (HD)	1.84	AEL	7.63	2.15
Heinrich et al. (1995)	2.5	AEL	8.40	2.35
Ishinishi et al. (1988) (LD)	2.32	AEL	9.75	2.75
Mauderly et al. (1987a)	7.08	AEL	10.9	3.05
Ishinishi et al. (1988) (HD)	3.72	AEL	15.8	4.4

[a]Effect levels are based on the critical effects of pulmonary histopathology and inflammation as reported in the individual studies.

[b]NOAEL: no-observed-adverse-effect level; LOAEL: lowest-observed-adverse-effect level; AEL: adverse-effect level; $BMCL_{10}$: lower 95% confidence estimate of the concentration of DPM associated with a 10% incidence of chronic pulmonary inflammation (inflam) or fibrosis (see Appendices A and B for more specifics).

[c]Lung burdens were derived from data generated from the animal portion of the Yu model using the concentration and duration scenario of each study. The human portion of the Yu model was then used to estimate the continuous, 70-year exposures that would result in this same lung burden, i.e., the HEC. See Table A-4 in Appendix A and accompanying text for further specifics on derivation.

[d]L/HD = light/heavy duty diesel engine.

[e]These values are the actual exposure levels used in the Nikula study. These values were converted into HEC and entered into BMC equations to obtain the estimate of the $BMCL_{10}$ listed. The lung burdens for the two $BMCL_{10}$s listed here were derived by interpolation.

mg/m^3 is estimated to result in a specific lung burden of 15.8 $\mu g/cm^2$. This latter lung burden is disproportionally elevated compared with the burden estimated to result from exposure to the lowest concentration. Applying the absolute ratio of lung burden/HEC at the lowest HEC exposure (i.e., 0.0587/ 0.032 = 1.8) to the highest concentration would result in a lower lung burden, 4.4 × 1.8 = 7.9 $\mu g/cm^2$, which is much lower than the 15.8 $\mu g/cm^2$ indicated. This disproportionate increase in lung burden as a function of DPM concentration would be predicted from the assumption in the Yu model that the overload phenomena occurs in humans, as is demonstrated in Figure 3-9 in Chapter 3. Inspection of Table 6-2 shows that this disproportion between lung burden and HEC begins to be noticeable around 0.33 mg/m^3, at the HEC derived from the Heinrich et al. (1995) study. HECs below this value are not appreciably influenced by the overload/disproportionate lung burden phenomenon.

Inspection of the combined interstudy dose-response continuum in Table 6-2 to elucidate a point of departure for an RfC entails some interpretation. Exposures at the lower end of this table show that elevated chronic exposures to DPM consistently result in AELs. Conversely, entries in the upper portion of this table show that low-level chronic exposures to DPM have minimal, if any, effects within the capability of these studies to detect them. Intermediate chronic exposures, from 0.128 mg/m^3 to 0.9 mg/m^3, are, however, less clear and effect levels and exposures either have no or few observable effects, or effects that are minimally adverse. In choosing from among levels (e.g., NOAELs, LOAELs, $BMCL_x$s) as a POD for derivation of an RfC, the methodology (U.S. EPA, 1994) provides guidance for choice of a highest no-effect level below an effect level; the interim guidance for the BMC suggests that for use as a point of departure, a benchmark (e.g., $BMCL_{10}$) should be within the range of the observable response data so as to avoid excessive extrapolation, and take the shape of the dose-response curve into consideration (Barnes et al., 1995; U.S. EPA, 1995). The highest no-effect HECs ($NOAEL_{HEC}$) in this table are 0.128 mg/m^3 and 0.144 mg/m^3 from the Ishinishi et al. (1988) study, nearly fivefold above other no-effect levels of 0.032 and 0.038 mg/m^3. The lower $BMCL_{10}$ (0.37 mg/m^3) is at nearly the same concentration as the lowest LOAEL of 0.33 mg/m^3 and thus may be too high an estimate for use as a POD based on these data. As discussed above, the limitations on this $BMCL_{10}$, including excessive extrapolation out of the observable range (see Appendix B for more specifics), make it a less than optimal candidate for consideration as a POD in the development of dose-response assessments and therefore was not used for this purpose in this assessment. However, this $BMCL_{10}$ (i.e., at a response rate of 0.1 or 10%) was generated directly from a modeled dose-response curve for chronic inflammation and lends credence to the other NOAELs in Table 6-2 as being associated with their respective dose-response curve at incidences of considerably less than 10%. Moreover, the HECs of less than 0.33 mg/m^3 are not appreciably influenced by the overload phenomenon (see above). Based on this analysis, the

value of 0.144 mg/m^3 is chosen as the POD for development of the RfC, because it is the highest $NOAEL_{HEC}$ among those available.

6.3.4. Uncertainty Factors (UF) for the RfC—A Composite Factor of 30

Areas of uncertainty designated in the RfC that are relevant to the DPM assessment are interindividual variability and animal-to-human extrapolation. Each shall be addressed in this section.

Considerable qualitative but little, if any, quantitative information exists regarding subgroups that could be sensitive to any respiratory tract effects of DPM. It is acknowledged that exposure to DPM could be additive to many other daily or lifetime exposures to airborne organic compounds and nondiesel ambient PM. It is also likely that individuals who predispose their lungs to increased particle retention through smoking or other high particulate burdens, who have existing respiratory tract inflammation or infections, or who have chronic bronchitis, asthma, or fibrosis could be more susceptible to adverse impacts from DPM exposure (U.S. EPA, 1996a, Chapter 5 of this document). Also, infants and children could have a greater susceptibility to the acute/chronic toxicity of DPM because of their greater breathing frequency and consequent potential for greater particle deposition in the respiratory tract, which has not reached full development. Increased respiratory symptoms and decreased lung function in children versus ambient PM levels, of which DPM is a part, have been observed (U.S. EPA, 1996a). Thus, even though the limited evidence currently available (see Chapter 5) produces no clear evidence that children are especially sensitive to effects from breathing DPM, the possibility that they actually may be more susceptible because of their inherent physiology and anatomy should remain a consideration. Likewise, a number of factors may modify normal lung clearance, including, aging, gender, and disease. It should be noted that the results of Mauderly et al. (1989) discussed in Chapter 5 indicated that rats with diseased lungs (emphysematous) were no more susceptible than rats with normal lungs to the effects of DE exposure. Although the exact role of these factors is not resolved, all would influence the particle dose to the lung tissue from inhalation exposure. Activity patterns related to occupation and habitation in the proximity of major roadways are certain to be contributory for some subgroups in receiving higher DPM exposures (Chapter 2). In the absence of DE-specific data, this assessment relies on a default UF value of 10 to account for possible interindividual human variability (U.S. EPA, 1994; Renwick and Lazarus, 1998).

Application of an animal-to-human extrapolation or interspecies uncertainty factor to an assessment may be modified via a number of circumstances. When the assessment is based on human data, no such UF is necessary. When the assessment is based on animal data, as is the case with DPM, a default UF of 10 typically is applied to the animal effect level. This latter action implies that the effect observed in the animal study would occur in humans at a 10-fold

lower concentration, ostensibly from some combination of pharmacokinetic and pharmacodynamic factors that would reflect greater dose (PK consideration) to the human target or greater sensitivity (PK consideration) of the human tissue.

The circumstances with DPM warrant modification away from application of the default UF for animal-to-human extrapolation. The first circumstance is the extensive effort in this assessment to address the pharmacokinetic component of the UF. The point of employing state-of-the-art lung dosimetry models with specific parameterization for DPM in conversion of animal exposures to human-equivalent exposures is to derive an estimate of interspecies pharmacokinetics; to know this aspect of interspecies difference with some degree of certainty. Having made this informed effort addresses a major portion of the PK component. It is acknowledged, however, that uncertainties about the model employed here (or any other model) persist. Although the model comparison shown in Chapter 3 indicates relatively minor variability in output among the various human models examined (see Table 3-3 and Figure 3-9) other sources of uncertainty and variability remain. These include, but are not limited to, matters such as the estimates if the model were applied to the general population or variability from the animal portion of the model(s). A second circumstance involves the pharmacodynamic or PD component of the interspecies UF, especially the aspect as to whether the experimental animal species used in the assessment is more or less sensitive than humans. In the consensus report of ILSI (2000) a specific recommendation is made concerning the PD aspect of the interspecies uncertainty factor for poorly soluble particles such as DPM. Because the pulmonary responses from DPM in the principal experimental species, the rat, are present under exposure conditions that do not appear to elicit any response in humans, the experimental species is considered more sensitive than humans. Accordingly, the report suggested that no accommodation be made for uncertainty concerning the pharmacodynamic component of the interspecies UF for DPM and presumably for any other PSP, as the rat appeared to be a sensitive species, more so even than the human. However, other information currently available on DPM suggests that, at least with regard to inflammatory effects, humans may indeed be as sensitive or even more so than rats. Section 5.1.1.1.3 discusses several studies where humans were exposed to airborne DPM and either precursors (Salvi et al., 2000; Nordenhall et al., 2000) or markers (Nightingale et al., 2000; Salvi et al.,1999) of inflammation were detected. These indicators of inflammation were in response to DPM levels of only 200–300 $\mu g/m^3$ of 1–2h duration. Note that in Table 6-2, NOAEL concentrations to which rats actually were exposed were only 100–400 $\mu g/m^3$, clearly within the range of the aforementioned human exposure levels. Thus, adverse effects (inflammation) have been shown to occur in humans at equivalent or possibly even lower levels of DPM than observed in rats, indicating that humans may indeed be at least as sensitive if not more so than rats.

The sum of these considerations on the animal-to-human UF is that, although major portions of uncertainty have been addressed, degrees of uncertainty persist in both the pharmacodynamic and pharmacokinetic components of the factor. In considering both this residual uncertainty and the information discussed above, it would be prudent to acknowledge partial degrees of uncertainty in both these areas with a partial uncertainty factor, i.e., $10^{0.5}$ vice 10^{1}, such that a factor of 3 would be applied for interspecies extrapolation.

In summary, the application of UFs for the two areas discussed above, interhuman and animal to human, would result in a composite uncertainty factor of 30, 10 for interhuman × 3 for animal to human. Use of other UFs, as discussed in the RfC methodology (U.S. EPA, 1994) for deficiencies in database or for duration extrapolation, is not considered necessary. It should be noted that, given the emerging research on DE-induced immunological effects, it may be necessary at a later date to reconsider the basis for selection of the critical effect and UFs and thus the entire derivation of the DE RfC.

6.3.5. Derivation of the RfC for Diesel Exhaust

On the basis of the above analysis, the value of 0.144 mg/m^3 DPM was selected as the point of departure for the RfC evaluation. This value was derived from concentrations in rat chronic studies that were modeled to obtain HECs. The pulmonary effects, histopathology and inflammation, were determined to be the critical noncancer effects. Response data on inflammation also were suggested by a specific scientific working group as a satisfactory surrogate for fibrogenic responses in assessing the pulmonary responses of poorly soluble particles such as DPM (ILSI, 2000). Sufficient documentation from other studies showed no effect in the portal-of-entry tissues, the extrathoracic (nasopharyngeal) region of the respiratory system, or in other organs at the lowest levels that produce pulmonary effects in chronic exposures. Application of the dosimetric model of Yu et al. (1991) to the exposure value from Ishinishi et al. (1988) of 0.46 mg/m^3 16 hr/day, 6 days/wk, a NOAEL, yielded a $NOAEL_{HEC}$ of 0.144 mg/m^3. Application of the composite UF yields the RfC:

$$NOAEL_{HEC} \div UF = RfC$$

$$0.144\ mg/m^3 \div 30 = 0.0048 mg/m^3 = 5\ \mu g/m^3.$$

6.4. EPIDEMIOLOGICAL EVIDENCE AND NAAQS FOR FINE PM

Historically, EPA has established primary NAAQS to protect sensitive human population groups against adverse health effects associated with ambient exposures to certain widespread air pollutants, including PM, ozone (O_3), carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and lead (Pb). The U.S. Clean Air Act (the Act) requires that EPA periodically review and revise as appropriate the criteria (scientific bases) and standards for each pollutant or class of pollutants (e.g., PM) for which NAAQS have been established. The primary, health-based NAAQS must be based on the latest scientific information useful in indicating the kind and extent of all effects on public health expected from the presence of the pollutant in the ambient air, which is evaluated in a "Criteria Document" (CD). The NAAQS are then set at levels that, in the judgment of the EPA Administrator, protect public health (as contrasted with the health of any individual) with an adequate margin of safety. In determining the degree of protection that will satisfy this mandate, EPA considers the nature and severity of the effects, the types of health evidence available, the kind and degree of scientific uncertainty that effects would in fact occur at any particular level of pollution, and the size and nature of sensitive populations at risk of experiencing exposures of concern. The EPA develops a staff paper to bridge the gap between the scientific criteria and the public health policy considerations the Administrator must take into account in reaching a final judgment. The EPA also must consider the recommendations of the Clean Air Scientific Advisory Committee (CASAC), an independent committee established by the Act specifically to advise the Administrator on air quality criteria and NAAQS. In contrast to an RfC, the NAAQS are not intended to identify a concentration that is protective against a hypothetical continuous lifetime exposure to a given level, but rather take into account expected actual exposure conditions of U.S. populations.

The original PM NAAQS were set in 1971 in terms of total suspended particulate matter (TSP) and included both inhalable and noninhalable particles, ranging in size up to 25–50 μm. A later periodic review of the PM criteria and NAAQS led to the setting in 1987 of PM_{10} NAAQS (150 $\mu g/m^3$, 24-h average; 50 $\mu g/m^3$, annual average) aimed at protecting against health effects associated with those inhalable particles capable of penetrating to lower (thoracic) regions of the human respiratory tract and depositing in tracheobronchial and alveolar tissue of the lung ($\leq$10.0 μm) (52 FR 24634, July 1, 1987). The most recently completed PM NAAQS review was based on an assessment of the latest available scientific information characterized in the EPA PM CD (U.S. EPA, 1996a) and additional staff assessments contained in an associated PM Staff Paper (U.S. EPA, 1996b). In 1997, on the basis of this information and taking into account CASAC recommendations and extensive public comments, EPA established new $PM_{2.5}$ NAAQS (15 $\mu g/m^3$, annual average; 65 $\mu g/m^3$, 24-h average) to protect against adverse health effects associated with exposures to fine PM. At the same time, EPA retained, in modified form,

the PM_{10} NAAQS originally set in 1987 to protect against effects associated with coarse fraction PM (62 FR 38652, July 18, 1997).[3]

The 1997 PM NAAQS decisions were based, in part, on important distinctions already highlighted by information present in the PM CD between the fine and coarse fractions of PM_{10} with regard to size, chemical composition, sources, and transport. Also of key importance were the assessment and interpretation of new epidemiological findings on health effects associated with ambient PM. The epidemiological evidence and basis for the NAAQS for fine PM are summarized below, followed by a discussion of the relevance of this information for noncancer assessment of DE.

6.4.1. Epidemiological Evidence for Fine PM

The PM CD (U.S. EPA, 1996a) and Staff Paper (U.S. EPA, 1996b) highlighted more than 80 newly published community epidemiologic studies, of which more than 60 found significant associations between increased mortality and/or morbidity risks and various ambient PM indicators. The main findings of concern were community epidemiology results showing ambient PM exposures to be statistically associated with increased mortality (especially among people over 65 years of age and those with preexisting cardiopulmonary conditions) and morbidity (indexed by increased hospital admissions, respiratory symptom rates, and decrements in lung function).

Time-series mortality studies reviewed in the 1996 PM CD (U.S. EPA, 1996a) provide strong evidence that ambient PM air pollution is associated with increases in daily human mortality and morbidity (e.g., increased hospital admissions and respiratory symptoms). These studies provided evidence that such effects occur at routine ambient PM levels, extending to 24-h concentrations below the 150 $\mu g/m^3$ level of the PM_{10} NAAQS set in 1987. Overall, as shown in Table 6-3, the PM_{10} effects estimates derived from the recent PM_{10} total mortality studies suggest that an increase of 50 $\mu g/m^3$ in 24-h average PM_{10} is significantly associated with an increase in total mortality, with an RR on the order of 1.025 to 1.05 in the general population. Table 6-3 also shows higher relative risks for increased hospital admissions for the elderly and for those with preexisting respiratory conditions, both of which represent subpopulations at special risk for mortality implications of acute exposures to air pollution, including PM; higher relative risks are also shown for increased respiratory symptoms and decreased lung function in children. Results are very similar over a range of statistical models used in the analyses, and are not artifacts of the methods by which the data were analyzed. Further, these studies suggest a possible linear,

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[3]At present, the 1997 $PM_{2.5}$ standards are the subject of ongoing litigation, although they legally remain in effect, as do the 1987 PM_{10} standards.

Table 6-3. Effect estimates per 50 μg/m³ increase in 24-h PM_{10} concentrations from U.S. and Canadian studies

Study location	RR (± CI) only PM in model	RR (± CI) other pollutants in model	Reported PM_{10} levels mean (min/max)*
Increased total acute mortality			
Six Cities[a]		—	
Portage, WI	1.04 (0.98, 1.09)	—	18 (±11.7)
Boston, MA	1.06 (1.04, 1.09)	—	24 (±12.8)
Topeka, KS	0.98 (0.90, 1.05)	—	27 (±16.1)
St. Louis, MO	1.03 (1.00, 1.05)	—	31 (±16.2)
Kingston/Knoxville, TN	1.05 (1.00, 1.09)	—	32 (±14.5)
Steubenville, OH	1.05 (1.00, 1.08)	—	46 (±32.3)
St. Louis, MO[c]	1.08 (1.01, 1.12)	1.06 (0.98, 1.15)	28 (1/97)
Kingston, TN[c]	1.09 (0.94, 1.25)	1.09 (0.94, 1.26	30 (4/67)
Chicago, IL[h]	1.04 (1.00, 1.08)	—	37 (4/365)
Chicago, IL[g]	1.03 (1.02, 1.04)	1.02 (1.01, 1.04)	38 (NR/128)
Utah Valley, UT[b]	1.08 (1.05, 1.11)	1.19 (0.96, 1.47)	47 (11/297)
Birmingham, AL[d]	1.05 (1.01, 1.10)	—	48 (21, 80)
Los Angeles, CA[f]	1.03 (1.00, 1.055)	1.02 (0.99, 1.036)	58(15/177)
Increased hospital admissions (for elderly > 65 yrs.)			
Respiratory Disease			
Toronto, CAN[i]	1.23 (1.02, 1.43)‡	1.12 (0.88, 1.36)‡	30-39*
Tacoma, WA[j]	1.10 (1.03, 1.17)	1.11 (1.02, 1.20)	37 (14, 67)
New Haven, CT[j]	1.06 (1.00, 1.13)	1.07 (1.01, 1.14)	41 (19, 67)
Cleveland, OH[k]	1.06 (1.00, 1.11)	—	43 (19, 72)
Spokane, WA[l]	1.08 (1.04, 1.14)	—	46 (16, 83)
COPD			
Minneapolis, MN[n]	1.25 (1.10, 1.44)	—	36 (18, 58)
Birmingham, AL[m]	1.13 (1.04, 1.22)	—	45 (19, 77)
Spokane, WA[l]	1.17 (1.08, 1.27)	—	46 (16, 83)
Detroit, MI°	1.10 (1.02, 1.17)	—	48 (22, 82)
Pneumonia			
Minneapolis, MN[n]	1.08 (1.01, 1.15)	—	36 (18,58)
Birmingham, AL[m]	1.09 (1.03, 1.15)	—	45 (19, 77)

Table 6-3. Effect estimates per 50 μg/m³ increase in 24-h PM_{10} concentrations from U.S. and Canadian studies (continued)

Study location	RR (± CI) only PM in model	RR (± CI) other pollutants in model	Reported PM_{10} levels mean (min/max)*
Spokane, WA[l]	1.06 (0.98, 1.13)	—	46 (16, 83)
Detroit, MI[o]	—	1.06 (1.02, 1.10)	48 (22, 82)
Ischemic HD			
Detroit, MI[p]	1.02 (1.01, 1.03)	1.02 (1.00, 1.03)	48 (22, 82)
Increased respiratory symptoms			
Lower Respiratory			
Six Cities[q]	2.03 (1.36, 3.04)	Similar RR	30 (13,53)
Utah Valley, UT[r]	1.28 (1.06, 1.56)[z]	—	46 (11/195)
	1.01 (0.81, 1.27)[π]		
Utah Valley, UT[s]	1.27 (1.08, 1.49)	—	76 (7/251)
Cough			
Denver, CO[x]	1.09 (0.57, 2.10)	—	22 (0.5/73)
Six Cities[q]	1.51 (1.12, 2.05)	Similar RR	30 (13, 53)
Utah Valley, UT[s]	1.29 (1.12, 1.48)	—	76 (7/251)
Decrease in Lung Function			
Utah Valley, UT[r]	55 (24, 86)**	—	46 (11/195)
Utah Valley, UT[s]	30 (10, 50)**	—	76 (7/251)
Utah Valley, UT[w]	29 (7.51)***	—	55 (1,181)

References:

[a]Schwartz et al. (1996a).
[b]Pope et al. (1992, 1994)/O_3.
[c]Dockery et al. (1992)/O_3.
[d]Schwartz (1993).
[f]Kinney et al. (1995)/O_3, CO.
[g]Ito and Thurston (1996)/O_3.
[h]Styer et al. (1995).
[i]Thurston et al. (1994)/O_3.
[j]Schwartz (1995)/SO_2.
[k]Schwartz et al. (1996b).
[l]Schwartz (1996).
[m]Schwartz (1994e).
[n]Schwartz (1994f).
[o]Schwartz (1994d).
[p]Schwartz and Morris (1995)/O_3, CO, SO_2.
[q]Schwartz et al. (1994).
[r]Pope et al. (1991).
[s]Pope and Dockery (1992).
[t]Schwartz (1994g)
[w]Pope and Kanner (1993).
[x]Ostro et al. (1991)
*Min/Max 24-h PM_{10} in parentheses unless noted otherwise as standard deviation (± S.D), 10 and 90 percentile (10, 90). NR = not reported.
[z]Children.
[π]Asthmatic children and adults.
**Means of several cities.
***PEFR decrease in ml/sec.
***FEV_1 decrease.
[‡]RR refers to total population, not just>65 years.

Source: Adapted from U.S. EPA, 1996b, Tables V-3, V-6, and V-7. See U.S. EPA (1996a,b) for all reference citations.

non-threshold PM/mortality relationship, but the data do not rule out the existence of an underlying nonlinear, threshold relationship (U.S. EPA, 1996a, 12-310-311; 1996b, VI-16). Figure 6-2 illustrates the consistency and coherence of the PM_{10} epidemiology findings for increased total and cause-specific mortality and morbidity risks in adults and children. In addition, Table 6-4 summarizes results from a wide array of U.S. and Canadian studies that showed increased risks of mortality and morbidity to be related to changes in short-term (24-h) fine PM (indexed by $PM_{2.5}$ and other fine particle indicators).

As summarized below, long-term exposure studies reviewed in the 1996 PM CD (U.S. EPA, 1996a) also provide evidence of associations between indicators of PM, including fine particle indicators, and chronic mortality and morbidity. Table 6-5 shows the direct comparisons of two key prospective studies of long-term PM mortality: the Harvard Six Cities Study (Dockery et al., 1993) and the American Cancer Society (ACS) Study (Pope et al., 1995). These two studies agree in their findings of strong associations between fine particles and increased mortality. The RR estimates for total mortality are large and highly significant in the Six Cities study. With their 95% confidence intervals, the RR estimate for a 50 $\mu g/m^3$ increase in $PM_{15,10}$ is 1.42 (1.16, 2.01), the RR estimate for a 25 $\mu g/m^3$ increase in $PM_{2.5}$ is 1.31 (1.11, 1.68), and the RR estimate for a 15 $\mu g/m^3$ increase in SO_4 is 1.46 (1.16, 2.16). The ACS study estimates for total mortality are smaller, but also more precise: RR = 1.17 (1.09, 1.26) for a 25 $\mu g/m^3$ increase in $PM_{2.5}$, and RR = 1.10 (1.06, 1.16) for a 15 $\mu g/m^3$ increase in SO_4. Both studies used Cox regression models and were adjusted for similar sets of individual covariates. In each case, however, caution must be applied in use of the stated quantitative risk estimates, given that the lifelong cumulative exposures of the study cohorts (especially in the dirtiest cities) included distinctly higher past PM exposures than those indexed by the more current PM measurements used to estimate long-term PM exposures in the study. Thus, somewhat lower relative risk estimates than the published ones may well apply. A third study by Abbey et al. (1991, 1995) reported no association between long-term PM exposure (indexed by TSP and other estimated PM indices) after 10 years, although the PM CD (U.S. EPA, 1996a) noted TSP may have been an inadequate index for exposure to inhalable particles and that additional follow-up might still reveal chronic effects.

An additional line of evidence concerning long-term effects may be seen in comparing cause-specific deaths in the Six Cities and ACS studies. The relative risks for the most versus the least polluted cities in the two studies are very similar for mortality from cardiopulmonary causes (U.S. EPA, 1996b, V-17). These two long-term exposure studies, taken together, suggest that there may be increases in mortality for specific disease categories that are consistent with long-term exposure to ambient fine particles. Moreover, at least some fraction of these deaths is

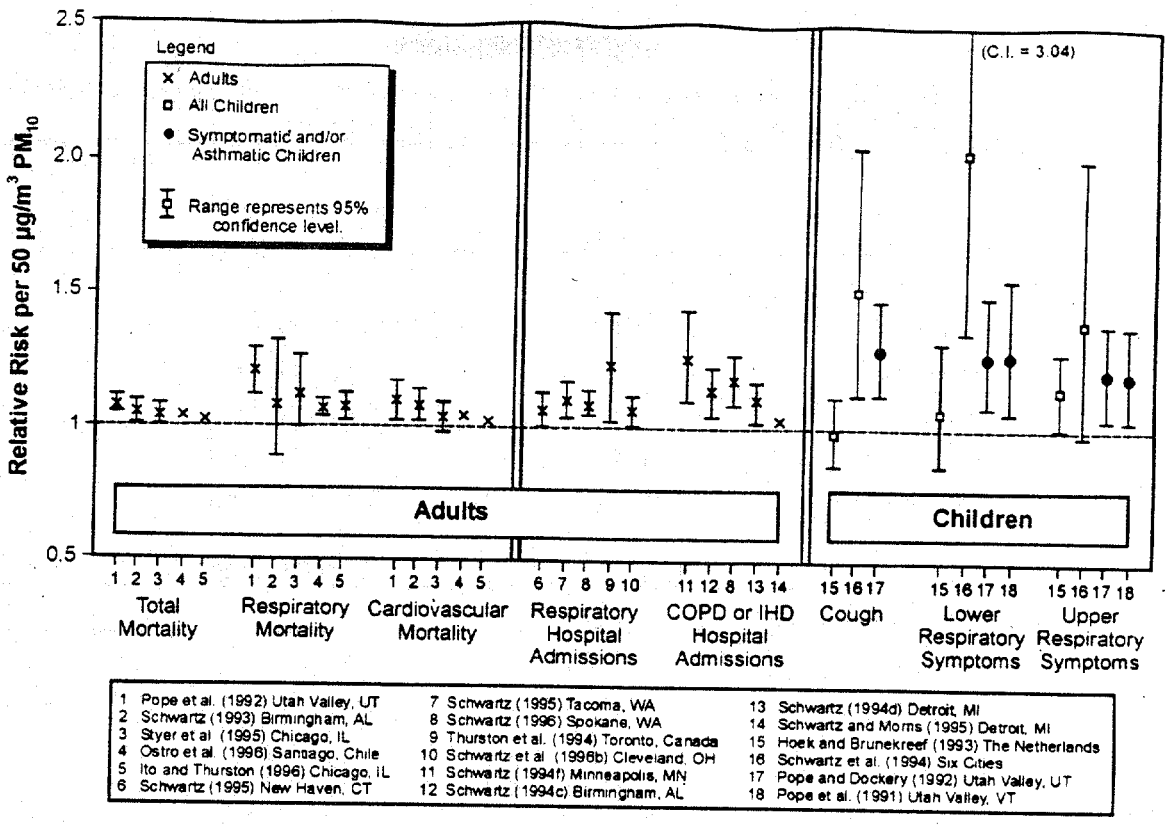

Figure 6-2. Relative risk (RR) estimates for increased mortality and morbidity endpoints associated with 50 µg/m³ increments in PM_{10} concentrations as derived from studies cited by numbers listed above each given type of health endpoint.

Note: Notice the consistency of RR elevations across studies for given endpoint and coherence of RR estimates across endpoints, e.g., higher RR values for symptoms versus hospital admissions and cause-specific mortality.

Source: PM Staff Paper (see U.S. EPA, 1996b for full reference citations for each study identified in figure.)

Table 6-4. Effect estimates per variable increments in 24-h concentrations of fine particle indicators ($PM_{2.5}$, $SO_4^=$, H^-) from U.S. and Canadian studies

Acute mortality	Indicator	RR (± CI) per 25 μg/m³ PM increase	Reported PM levels mean (min/max)*
Six Cities[a]			
Portage, WI	$PM_{2.5}$	1.030 (0.993, 1.071)	11.2 (±7.8)
Topeka, KS	$PM_{2.5}$	1.020 (0.951, 1.092)	12.2 (±7.4)
Boston, MA	$PM_{2.5}$	1.056 (1.038, 1.0711)	15.7 (±9.2)
St. Louis, MO	$PM_{2.5}$	1.028 (1.010, 1.043)	18.7 (±10.5)
Kingston/Knoxville, TN	$PM_{2.5}$	1.035 (1.005, 1.066)	20.8 (±9.6)
Steubenville, OH	$PM_{2.5}$	1.025 (0.998, 1.053)	29.6 (±21.9)
Increased hospitalization			
Ontario, CAN[b]	$SO_4^=$	1.03 (1.02, 1.04)	R = 3.1-8.2
Ontario, CAN[c]	$SO_4^=$	1.03 (1.02, 1.04)	R = 2.0-7.7
	O_3	1.03 (1.02, 1.05)	
NYC/Buffalo, NY[d]	$SO_4^=$	1.05 (1.01, 1.10)	NR
Toronto[d]	H^- (Nmol/m³)	1.16 (1.03, 1.30)*	28.8 (NR/391)
	$SO_4^=$	1.12 (1.00, 1.24)	7.6 (NR, 48.7)
	$PM_{2.5}$	1.15 (1.02, 1.78)	18.6 (NR, 66.0)
Increased respiratory symptoms			
Southern California[e]	$SO_4^=$	1.48 (1.14, 1.91)	R = 2-37
Six Cities[f] (Cough)	$PM_{2.5}$	1.19 (1.01, 1.42)**	18.0 (7.2, 37)***
	$PM_{2.5}$ Sulfur	1.23 (0.95, 1.59)**	2.5 (3.1, 61)***
	H^-	1.06 (0.87, 1.29)**	18.1 (0.8, 5.9)***
Six Cities[f] (Lower Resp. Symp.)	$PM_{2.5}$	1.44 (1.15-1.82)**	18.0 (7.2, 37)***
	$PM_{2.5}$ Sulfur	1.82 (1.28-2.59)**	2.5 (0.8, 5.9)***
	H^-	1.05 (0.25-1.30)**	18.1 (3.1, 61)***
Decreased lung function			
Uniontown, PA[g]	$PM_{2.5}$	PEFR 23.1 (-0.3, 36.9) (per 25 μg/m³)	25/88 (NR/88)

References:
[a]Schwartz et al. (1996a)
[b]Burnett et al. (1994)
[c]Burnett et al. (1995) O_3
[d]Thurston et al. (1992, 1994)
[e]Ostro et al (1993)
[f]Schwartz et al. (1994)
[g]Neas et al. (1995)

*Min/Max 24-h PM indicator level shown in parentheses unless otherwise noted as (± S.D.), 10 and 90 percentile (10,90) or R = range of values from min-max, no mean value reported.
*Change per 100 nmoles/m³.
**Change per 20 μg/m³ for $PM_{2.5}$; per 5 μg/m³ for $PM_{2.5}$ sulfur; per 25 nmoles/m³ for H^-.
***50th percentile value (10,90 percentile).

Source: Adapted from U.S. EPA, 1996b, Table V-12. See U.S. EPA (1996a,b) for all reference citations.

Table 6-5. Effect estimates per increments[a] in annual average levels of fine particle indicators from U.S. and Canadian studies

Type of health effect and location	Indicator	Change in health indicator per increment in PM[a]	Range of city PM levels mean ($\mu g/m^3$)
Increased total chronic mortality in adults		**Relative risk (95% CI)**	
Six City[b]	$PM_{15/10}$	1.42 (1.16-2.01)	18-47
	$PM_{2.5}$	1.31 (1.11-1.68)	11-30
	$SO_4^=$	1.46 (1.16-2.16)	5-13
ACS Study[c] (151 U.S. SMSA)	$PM_{2.5}$	1.17 (1.09-1.26)	9-34*
	$SO_4^=$	1.10 (1.06-1.16)	4-24
Increased bronchitis in children		**Odds ratio (95% CI)**	
Six City[d]	$PM_{15/10}$	3.26 (1.13, 10.28)	20-59
Six City[e]	TSP	2.80 (1.17, 7.03)	39-114
24 City[f]	H^-	2.65 (1.22, 5.74)	6.2-41.0
24 City[f]	$SO_4^=$	3.02 (1.28, 7.03)	18.1-67.3
24 City[f]	$PM_{2.1}$	1.97 (0.85, 4.51)	9.1-17.3
24 City[f]	PM_{10}	3.29 (0.81, 13.62)	22.0-28.6
Southern California[g]	$SO_4^=$	1.39 (0.99, 1.92)	—
Decreased lung function in children			
Six City[d,h]	$PM_{15/10}$	NS Changes	20-59
Six City[e]	TSP	NS Changes	39-114
24 City[i,j]	H^- (52 nmoles/m^3)	-3.45% (-4.87, -2.01) FVC	—
24 City[i]	$PM_{2.1}$ (15 $\mu g/m^3$)	-3.21% (-4.98, -1.41) FVC	—
24 City[i]	$SO_4^=$ (7 $\mu g/m^3$)	-3.06% (-4.50, -1.60) FVC	—
24 City[i]	PM_{10} (17 $\mu g/m^3$)	-2.42% (-4.30, -.0.51) FVC	—

[a]Estimates calculated annual-average PM increments assume: a 100 $\mu g/m^3$ increase for TSP; a 50 $\mu g/m^3$ increase for PM_{10} and PM_{15}; a 25 $\mu g/m^3$ increase for $PM_{2.5}$; and a 15 $\mu g/m^3$ increase for $SO_4^=$, except where noted otherwise; a 100 nmole/m^3 increase for H^-.

[b]Dockery et al. (1993).
[c]Pope et al. (1995).
[d]Dockery et al. (1989).
[e]Ware et al. (1986).
[f]Dockery et al. (1996).
[g]Abbey et al. (1995a,b,c).
[h]NS Changes = No significant changes.
[i]Raizenne et al. (1996).
[j]Pollutant data same as for Dockery et al. (1996).
*Range of annual median values for subset of 50 cities.

Source: Adapted from U.S. EPA, 1996a, Table 12-6 and U.S. EPA, 1996b, Table V-8. See U.S. EPA (1996a,b) for all reference citations.

likely to be a consequence of cumulative, long-term exposure effects. These effects extend beyond the additive impacts of short-term exposure episodes, in terms of producing marked increases above the expected number of daily deaths among especially susceptible groups, such as the elderly and those with pulmonary disease.

The PM CD (U.S. EPA, 1996a) also highlighted a growing body of evidence directly comparing fine and coarse fraction PM effects that suggests that fine particles are more strongly related than coarse fraction particles to increased mortality and morbidity in both short- and long-
term exposure studies. Such evidence notably includes the results of analyses of the type illustrated in Figure 6-3 through 6-5. More specifically, Figure 6-3 shows a stronger relationship between changes in short-term (24-h) concentrations of fine particles (indexed by $PM_{2.5}$) and increased mortality risks than for changes in short-term concentrations of coarse fraction particles (indexed by $PM_{15-2.5}$). Similarly, a stronger relationship is seen between chronic mortality and long-term exposure to fine particles (including both the sulfate and nonsulfate components) than exposure to coarse fraction particles (Figure 6-4), and a much stronger relationship between lung function decrements and long-term exposure to fine particles than to coarse fraction particles (Figure 6-5).

6.4.2. NAAQS for Fine PM

The health effects evidence discussed above is relevant to this current HAD, as both this document (Chapter 2) and the PM CD present information that clearly shows DPM to be a constituent of ambient fine particles. Therefore, it is reasonable to conclude that DPM is associated, but to an undetermined degree, with the health effects described above. Whereas broader public health factors are taken into account in setting NAAQS than are relevant for this noncancer assessment of lifetime exposure for DPM, the annual $PM_{2.5}$ NAAQS based primarily on this evidence is of interest in considering the extent to which the RfC for DE (as derived above in Section 6.3) is concordant with the information on fine particles.

As presented in the Federal Register final rule notice (62 FR 38652, July 18, 1997), EPA drew upon the quantitative epidemiology information concisely summarized above to derive a rationale for selection of an annual-average $PM_{2.5}$ standard.[4] First, to appropriately reflect the

[4]As an initial matter, EPA concluded that the existing PM_{10} standards were not adequate to protect public health, that fine and coarse fraction particles should be considered separately, that $PM_{2.5}$ was the appropriate indicator to use for fine particles, and that an annual $PM_{2.5}$ standard could provide the requisite reduction in risk associated with both annual and 24-h averaging times in most areas of the United States. This annual standard,

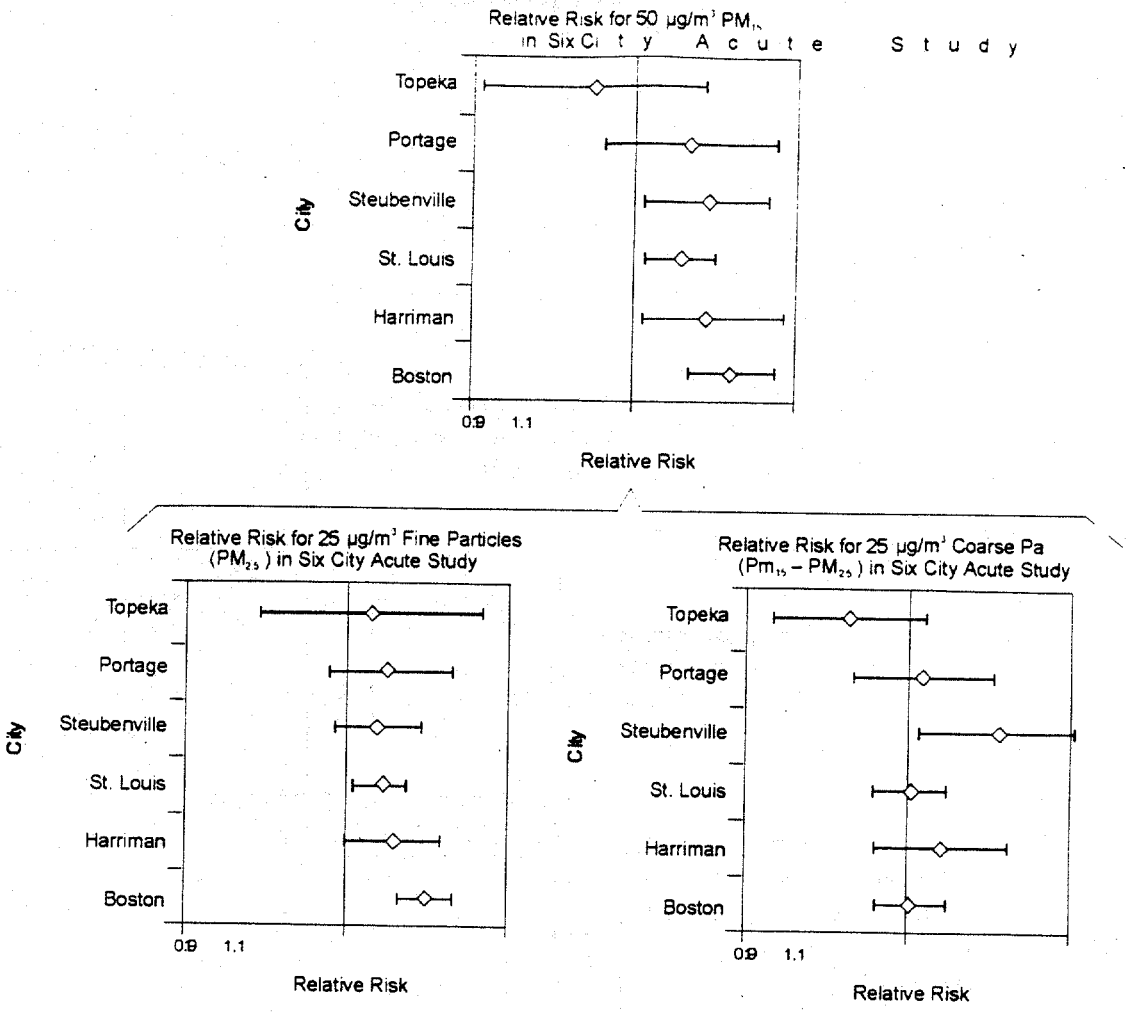

Figure 6-3. Relative risks of acute mortality in Harvard Six Cities Study, for inhalable thoracic particles (PM_{15}/PM_{10}), fine particles ($PM_{2.5}$), and coarse fraction particles (PM_{15}-$PM_{2.5}$).

Note: The coarse fraction effects are smaller and statistically nonsignificant (i.e., lower 95% confidence intervals do not exceed relative risk of 1.0), except in Steubenville where there is high correlation between fine and coarse particles ($R^2 = 0.69$).

Source: PM CD (U.S. EPA, 1996a) graphical depiction of results from Schwartz et al. (1996).

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together with a 24-h standard, could provide supplemental protection against extreme peak fine particle levels that might occur in some localized situations or in areas with distinct variations in seasonal fine particle levels (62 FR 38652).

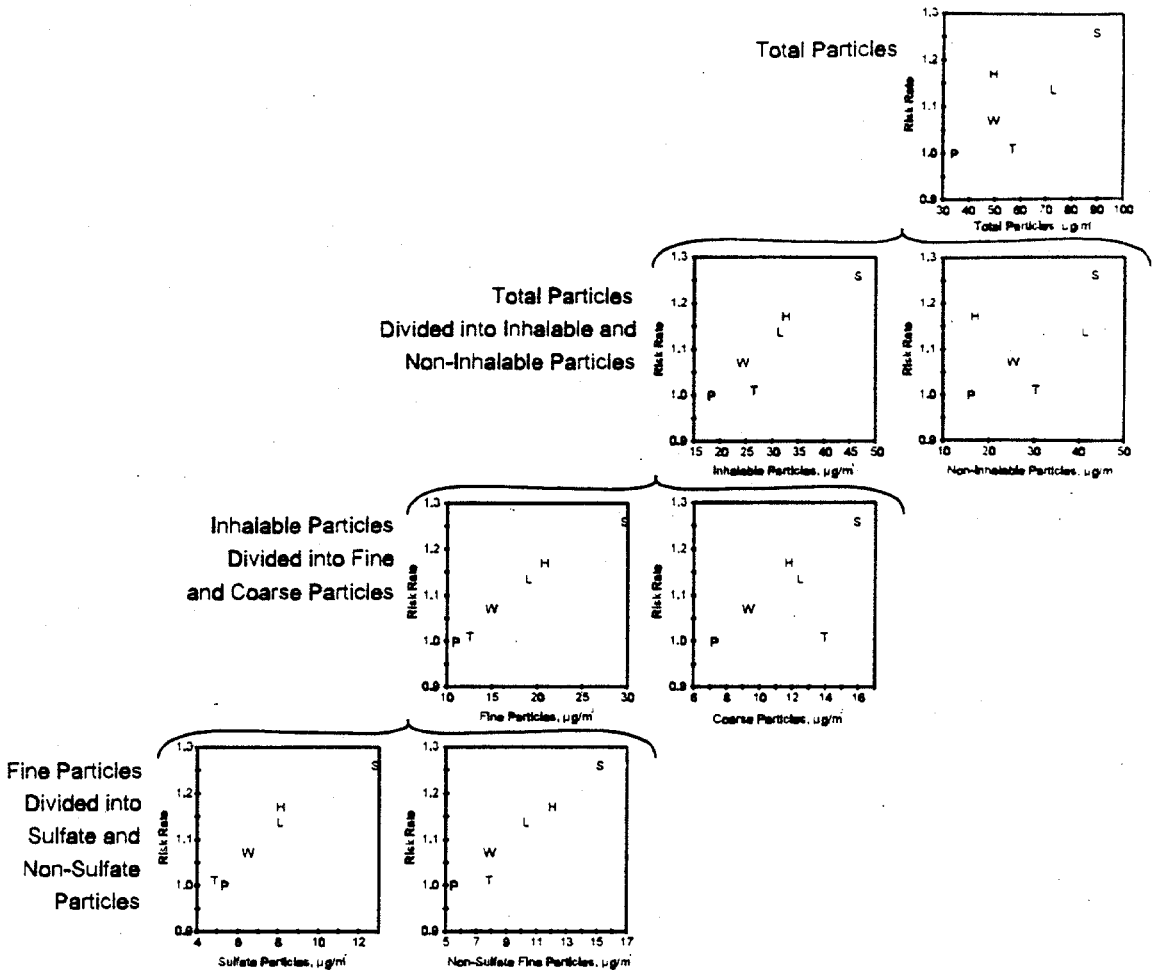

Figure 6-4. Adjusted relative risks for mortality are plotted against each of seven long-term average particle indices in the Harvard Six Cities Study, from largest range (total suspended particles, upper right) through sulfate and nonsulfate fine particle concentrations (lower left).

Note: A relatively strong linear relationship is seen for fine particles, and for sulfate and nonsulfate components. Topeka, which has a substantial coarse particle component of inhalable (thoracic) particle mass, stands apart from the linear relationship between relative risk and inhalable particle concentration.
Source: U.S. EPA (1996a) replotting of results from Dockery et al. (1993).

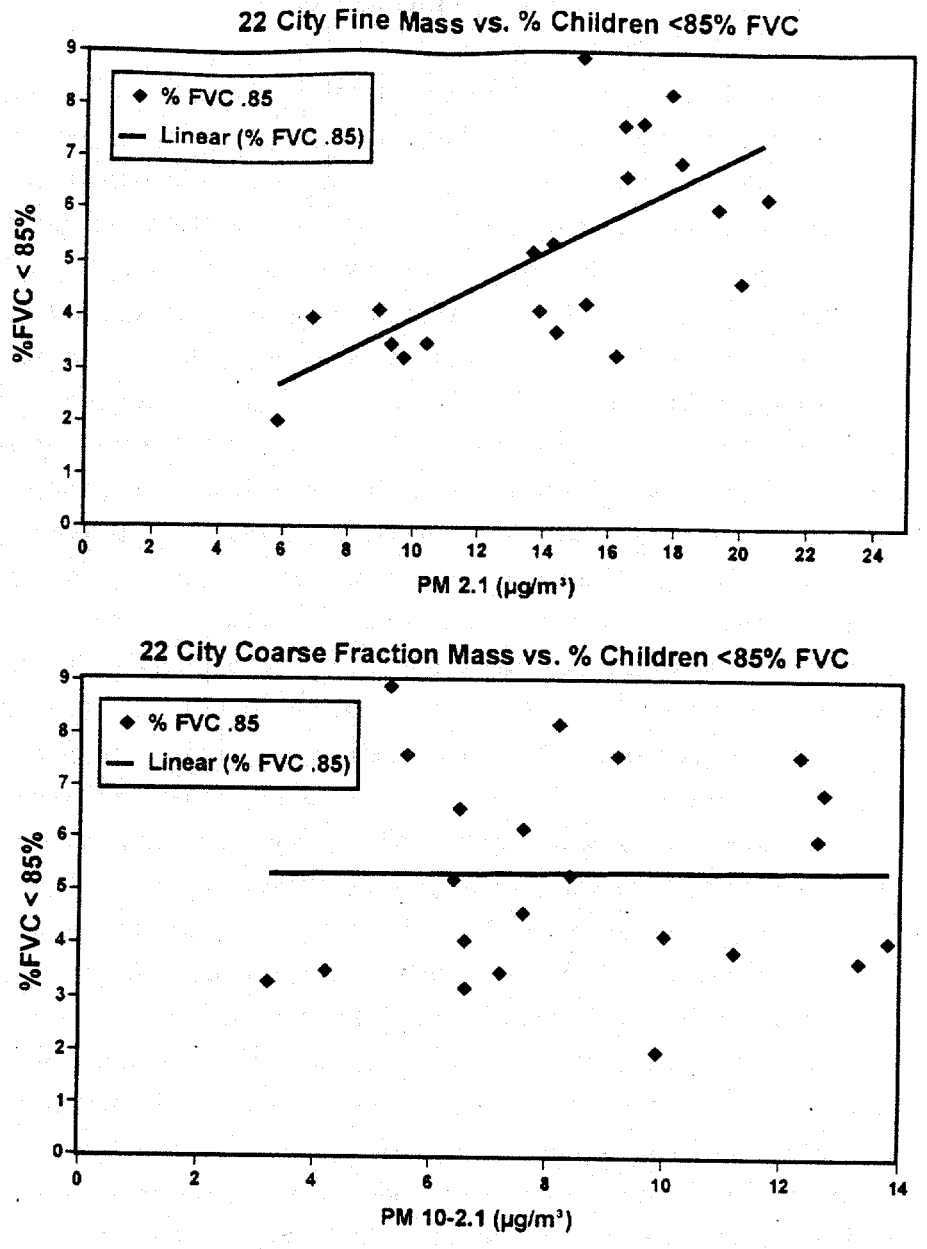

Figure 6-5. Percent of children with <85% normal FVC versus annual-average fine ($PM_{2.1}$) particle concentrations and coarse fraction ($PM_{10-2.1}$) levels for 22 North American cities.

Note: A much stronger connection appears between fine particles and lung function decrements (top panel) than for coarse fraction particles (bottom panel).
Source: PM Staff Paper (1996b) graphical depiction of results from Razienne et al. (1996).

weight of evidence as a whole, EPA concluded that it was appropriate to limit annual $PM_{2.5}$ concentrations to somewhat below those where the body of epidemiological evidence is most consistent and coherent, recognizing both the strengths and limitations of the full range of information on the health effects of PM, as well as associated uncertainties. In accordance with EPA staff and CASAC views on the relative strengths of the epidemiologic studies, major reliance was placed on several short-term (24-h) exposure studies showing significantly increased risks of daily mortality (Schwartz et al., 1996) and morbidity indexed by hospital admissions (Thurston et al., 1994) and respiratory symptoms/lung function decrements in children (Schwartz et al., 1994; Neas et al., 1995) in relationship to increased fine particle ($PM_{2.5}$) concentrations. Whereas it was recognized that health effects may occur over the full range of concentrations observed in these studies, it was concluded that the strongest evidence for short- term $PM_{2.5}$ effects occurs at concentrations near the long-term (e.g., annual) average. More specifically, the strength of the evidence of effects increases for concentrations of $PM_{2.5}$ that are at or above the long-term mean levels reported for these studies. Given the serious nature of the potential effects, EPA judged that it was both prudent and appropriate to select a level for an annual standard at or below such concentrations. More specifically, statistically significant increases in relative risks for daily mortality or morbidity were most clearly observed in these studies to be associated with 24-h fine particle concentrations in cities with long-term mean fine particle concentrations ranging from about 16 to about 21 $\mu g/m^3$, leading to the judgment that an annual standard level of 15 $\mu g/m^3$ would be appropriate.

Before reaching a final conclusion, the epidemiologic studies of long-term exposures to fine particles were also considered, which may reflect the accumulation of daily effects over time as well as potential effects uniquely associated with long-term exposures. Even subject to additional uncertainties, these studies were judged to provide important insights with respect to the overall protection afforded by an annual standard. In particular, the annual mean $PM_{2.5}$ concentrations for the multiple cities included in the two key long-term exposure mortality studies (Dockery et al., 1993; Pope et al., 1995) were 18 $\mu g/m^3$ and about 21–22 $\mu g/m^3$, respectively, with most of the 50 cities in the Pope, et al. (1995) having mean $PM_{2.5}$ concentrations above 15 $\mu g/m^3$. Taken together with other long-term exposure studies and considering other factors discussed in the final rule (62 FR 38676, July 18, 1997), EPA concluded that the concordance of evidence for PM effects and associated levels provides clear support for an annual standard set at 15 $\mu g/m^3$.

6.4.3. DPM as a Component of Fine PM

Chapter 2 of this document, as well as the PM CD (U.S. EPA, 1996a), report the extent to which DPM may contribute to ambient $PM_{2.5}$ concentrations. In some urban situations, the annual average fraction of $PM_{2.5}$ attributable to DPM (according to mass concentrations) is about 35% on the high end, although the proportion appears to be more typically in the range of about 10% (see Chapter 2, Table 2-23 and Section 2.4.2.1).

An approach to considering the relationship of toxicity between DPM and $PM_{2.5}$ would be simply to assume that, as DPM is contributory to the content of ambient $PM_{2.5}$, so too would it be contributory to toxicity of $PM_{2.5}$. This approach is qualitative only because no firm basis currently exists for apportioning toxicity among the various components of $PM_{2.5}$. Nevertheless, some qualitative information from laboratory animal studies does exist, showing that DPM is no more potent at eliciting pulmonary pathology than other poorly soluble particles such as talc, titanium dioxide, or carbon black in rats, or talc or titanium dioxide in mice. No data suggest that DPM is any more potent in eliciting pulmonary pathology than any other poorly soluble particle that typically may be present in ambient $PM_{2.5}$. It may be reasonable to suggest, then, that DPM is no more likely to be toxicologically potent than any other fine particle constituents that typically make up ambient $PM_{2.5}$.

Based on the foregoing aspects of such an approach, a conclusion could be drawn that as long as DPM constituted its current approximate proportion to $PM_{2.5}$, the annual $PM_{2.5}$ standard would also be expected to provide a measure of protection for DPM. Even if a basis did exist to apportion toxicity among the various components of ambient $PM_{2.5}$, such as DPM, use of such information in an approach to derive a safe air level for DPM would result in only a generalized, nonspecific estimate limited by a variety of factors including the accuracy of the apportionment of DPM from $PM_{2.5}$. The RfC derived in Section 6.3 was based on an approach that utilized toxicological information from actual DPM exposures, a more direct approach that would result in a more specific estimate not limited by any apportionment scheme.

6.5. CHARACTERIZATION OF THE NONCANCER ASSESSMENT FOR DIESEL EXHAUST

Adverse health effects from short-term acute (high-level) exposures to DE such as occupational reports of decreases in lung function, wheezing, chest tightness, increases in airway resistance, and reports in laboratory animals of inflammatory airway changes and lung function changes are acknowledged but are not assessed quantitatively. The focus of this dose-response assessment is on the adverse noncancer health consequences of a lifetime, low-level, continuous air exposure by humans to DE.

This assessment uses the whole particle, termed DPM, as the key index or measure of DE dose. DPM includes any and all adsorbed organics, among which are a large number of PAHs, heterocyclic compounds, and their derivatives (Chapter 2), as well as the carbon core. It is not possible to separate the carbon core of DPM from the adsorbed organics to compare the toxicity in exposures other than with limited in-vitro-type scenarios. The dosimetric model used in the derivation of the RfC (Yu et al., 1991) is consistent with this designation, as it considers DPM as well as the adsorbed organics as two types, slow-cleared and fast-cleared. Studies with diesel do occasionally report levels of accompanying gaseous components of DE (e.g., NO_x, CO), but nearly all report particle concentration and characteristics.

Adverse responses occurring in the rat lung have been used in this assessment as the basis for characterizing nonneoplastic human lung responses, yet use of these data in hazard evaluation for cancer is not considered relevant to humans. The basis for this use of these noncancer pulmonary effects in rats for derivation of an RfC includes the fact that humans and rats exhibit similar responses to other poorly soluble particles and also that similar noncancer effects are seen in other species (ILSI, 2000; Freedman and Robinson, 1988). Thus, when viewed across species (including humans), the nonneoplastic pulmonary effects of inflammation and fibrosis used in this assessment are dissociable from the cancer response and are of likely relevance to humans.

As a part of the RfC methodology (U.S. EPA, 1994), dose-response assessments are assigned levels of confidence that are intended to reflect the strengths and limitations of an assessment as well as to indicate the likelihood of the assessment changing with any additional information. Confidence levels of either low, medium, or high are assigned both to the study (or studies) used in the assessment to characterize the critical effects and to the overall toxicological database of the substance. An overall confidence level also is assigned to the entire assessment. Usually, it is the same, or in any case no higher than the level assigned to the database.

Compared with the databases of most other toxicants, the basic toxicological database for DE is substantial. The critical effects are characterized using not one but multiple long-term chronic studies conducted independently of one another (Tables 6-1 and 6-2). The exhaustive manner in which these studies were conducted and reported also imparts a high degree of confidence. Both developmental and reproductive areas are addressed. Also, ancillary studies that address mechanistic aspects of DE toxicity, either as the whole particle with adsorbed organics, or segregated as a poorly soluble particle and extracted organics, are available and used in this assessment. Although only limited human data are available, extensive consideration has been given to the relevancy of the animal studies to the human condition. On the other hand, data from related toxicants such as general ambient PM indicate effects in endpoints (e.g.,

cardiovascular measures) that have not been addressed in the DPM database. A major point to consider in assigning confidence in this assessment, and a reason that the value of the RfC may change in the future, is the emerging issue of allergenicity caused or exacerbated by DE. Although information to evaluate allergenicity in parallel to the present effects (pulmonary inflammation and histopathology) is currently lacking, future efforts to elucidate and characterize this effect may well be a driver to make a reevaluation of the noncancer RfC derivation for DE appropriate. With respect to the current RfC for DE, the confidence level is medium, both for the database and overall. The level reflects the relevance of (and information lacking on) allergenicity effects associated with DE in humans, and the possibility that the current RfC could change as a consequence of this information becoming available from the scientific community.

In the introductory portion of this chapter, DPM is acknowledged as a constituent of ambient PM (U.S. EPA, 1996a,b). A discussion of the quantitative epidemiology, particularly regarding fine PM, indicated that public health effects, including premature mortality, increased hospital admissions, respiratory symptoms, and decreased lung function, were observed in populations living in areas with long-term mean $PM_{2.5}$ levels generally ranging above 15 $\mu g/m^3$. Application of the RfC method, which involved critical consideration of the entirety of the disparate DE database with many chronic studies from several different species, evaluation of a myriad of possible DE-specific toxicological endpoints, and use of extrapolation models, produced a value of 5 $\mu g/m^3$. As the accuracy of the RfC is stated in the definition (*"...within an order of magnitude ..."*), this dose-response estimate could be considered to be not different from the level of 15 $\mu g/m^3$, the lower end of the range identified for $PM_{2.5}$. It is acknowledged here again that the levels of the $PM_{2.5}$ NAAQS should not be considered as indicative of the same degree of health protection for DE as intended by the RfC. Nevertheless, the congruence of these estimates tends to enhance the overall confidence that this range of levels is near or inclusive of those that would be expected to be protective of the human population against the health effects of DE.

6.6. SUMMARY

Table 6-6 summarizes the key data and factors used in the dose-response analysis leading to the derivation of the RfC for DE. The DE RfC of 5 μg DPM/m^3 is a chronic exposure likely to be without an appreciable risk of adverse human health effects.

The link between ambient fine PM and DPM with respect to origin, content, and possible health effects has been presented and discussed in this chapter, and the general congruence between the DE RfC and the level of the annual NAAQS for fine particles has been noted.

Although these values should not be compared directly, it is reasonable to observe that the annual $PM_{2.5}$ standard would be expected to provide a measure of protection for DPM, reflecting

Table 6-6. Decision summary for the quantitative noncancer RfC assessment for continuous exposure to diesel particulate matter (DPM)

Quantitative assessment for noncancer effects from lifetime exposure to DPM	5 μg/m³
Critical effect	Pulmonary inflammation and histopathology in rats
Principal study	Array of four chronic rat studies
Designated basis for quantitation (exposures in rats)	0.46 mg DPM /m³, 16 hr/day, 6 d/wk, 130 wks; a NOAEL
$NOAEL_{HEC}$ (HEC)	0.144 mg DPM / m³
Adjustments for uncertainty factors (interspecies variability and intraspecies extrapolation)	30
$NOAEL_{HEC}/UF = RfC$	0.144 mg/m³ / 30 = 5 μg/m³

DPM's current approximate proportion to $PM_{2.5}$.

The estimated air concentration of 5 μg/m³ (the RfC, a lifetime exposure to DE measured as DPM) is above the ambient air levels reported in most rural areas but could be below those levels reported under short-term conditions in some urban scenarios, such as at busy intersections or bus stops (see Chapter 2, Table 2-23). The RfC is intended to address lifetime chronic exposures and aspects of time-averaging for less than lifetime scenarios, such as, for example, acute exposures at busy intersections or bus stops, which are not addressed in this particular assessment.

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of what is seen in the ambient PM data is of interest, as epidemiologic evidence for an effect of ambient PM on lung cancer mortality or incidence could possibly contribute to evaluation of DE-specific epidemiologic data.

Chapters 2 and 5 noted that DPM, consisting mostly of fine particles (<1.0 mm diameter), represents a toxicologically important component of typical ambient fine particle mixes. As discussed in Chapter 6, several large-scale prospective studies (Harvard Six Cities Study; American Cancer Society (ACS) Study; Adventist Health Study of Smog (AHSMOG) provide important evidence regarding associations between chronic exposures to ambient fine particles and increased risks of noncancer mortality/morbidity effects (e.g., cardiorespiratory-related deaths or hospital admissions) (U.S. EPA, 1996a). As summarized below, these same studies also evaluated relationships between chronic PM exposures and lung cancer mortality and/or incidence.

As an initial matter, both the Harvard Six Cities Study (Dockery et al., 1993), of approximately 8,000 adults in six cities comprising a transect across the northcentral and northeastern United States, and the ACS Study (Pope et al., 1995), of 550,000 adults in 151 cities across all U.S. geographic regions, found markedly increased relative risks (RR) of lung cancer mortality associated with smoking. More specifically, the Six City Study reported increased risks of smoking for current (RR = 8.00, 95% CI = 2.97-21.6) and former (RR = 2.54, CI = 0.90-7.18) smokers, with the ACS Study reporting striking similar increased risks for current smokers (RR = 9.73, 95% CI = 5.96-15.9).

After controlling for smoking and other risk factors, both the Six Cities Study and the ACS Study (using a subset of 50 of the 151 cities) evaluated relationships between long-term exposure to fine PM (indexed by $PM_{2.5}$), from the least to the most polluted of the cities in each study, and lung cancer mortality. In both studies, lung cancer mortality risks were not statistically significantly associated with ambient $PM_{2.5}$ concentrations in combined analyses of data for both males and females (RR = 1.37, 95% CI = 0.81-2.31, in the Six Cities Study; RR = 1.03, 95% CI = 0.80-1.33, in the ACS Study). Also, lung cancer mortality risks were not statistically significantly associated with ambient $PM_{2.5}$ concentrations in the ACS Study for smaller sample size subgroups broken out by sex and smoking status. In addition, analyses of data from the AHSMOG series of studies, of 6,338 nonsmoking long-term California adult residents, found no statistically significant associations between $PM_{2.5}$ (estimated from visibility data) and lung cancer mortality or total mortality (Abbey et al., 1995); further, no such associations were reported for PM_{10} (estimated from total suspended particulate matter [TSP] data) in the same study. Earlier AHSMOG analyses (Abbey et al., 1991) reported no statistically significant associations between TSP (which includes not only fine PM but also larger coarse-

7. CARCINOGENICITY OF DIESEL EXHAUST

7.1. INTRODUCTION

Initial health hazard concerns regarding the potential carcinogenicity of diesel engine exhaust (DE) 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 epidemiologic 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 retrospective cohort and case-control studies have been carried out with railroad workers, dockworkers, truck drivers, construction workers, miners, and bus garage employees. During 1986 and 1987, several chronic animal cancer bioassays were published. These studies and numerous laboratory investigations carried out since then have been directed toward assessing the carcinogenic potential of whole exhaust, evaluating the importance of various exhaust components in the induction of cancer, and understanding the mode of action and implications of deposition, retention, and clearance of DE particles.

7.1.1. Overview

This chapter evaluates the carcinogenic potential of DE in both humans (Section 7.2) and animals (Section 7.3), discusses mode(s) of action (Section 7.4), and provides an overall weight-of-evidence evaluation (Section 7.5) for carcinogenicity in humans. This chapter also summarizes evaluations of DE conducted by other organizations (Section 7.6) and the final conclusions (Section 7.7) identify major uncertainties for which additional research is needed. This assessment focuses on DE, although it should be noted that diesel particles make up a portion of ambient particulate matter (PM) (Chapter 2, Section 2.2.3; Chapter 6, Section 6.4.3), and thus, the ambient PM data may have some relevance.

7.1.2. Ambient PM-Lung Cancer Relationships

A brief overview of the data regarding exposure to ambient PM and lung cancer is provided as background information and is based on analyses contained in the 1996 Air Quality Criteria for PM (PM CD) (U.S. EPA, 1996a).[1] With DE being part of ambient PM, the question

[1]As noted in Chapter 6, a new PM CD is now being prepared to reflect the latest scientific studies on ambient PM available since the last document was completed.

(Garland et al., 1988), gastrointestinal cancer (Balarajan and McDowall, 1988; Guberan et al., 1992), and prostate cancer (Aronsen et al., 1996). A detailed review of 22 lung cancer studies is presented in this section; a few more studies exist, but these 22 are judged to be the key ones. A detailed review of other health effect studies is not presented because findings are equivocal.

Excess risk of bladder cancer has been reported in several studies (Howe et al., 1980; Wynder et al., 1985; Hoar and Hoover et al., 1985; Silverman et al., 1983; Vineis and Magnani 1985; Silverman et al., 1986; Jensen et al., 1987; Steenland et al., 1987; Isocovich et al., 1987; Risch et al., 1988; Iyer et al., 1990; Steineck et al., 1990; Cordier et al., 1993; Notani et al., 1993). Very few studies found significant excesses after adjustment for cigarette smoking. Most studies failed to show any association between exposure to DE and occurrence of bladder cancer. Some authors have reported excess mortality from lymphohematopoietic system cancers in people potentially exposed to diesel fumes. Rushton and Alderson (1983) and Howe and Lindsay (1983) found increased mortality from lymphatic neoplasms. Balarajan and McDowall (1983) found raised mortality for malignant lymphomas. Flodin et al. (1987) observed increased risk for multiple myeloma, and Bender et al. (1989) reported excess mortality from leukemia. Because evidence for bladder cancer and lymphohematopoietic cancer was found to be equivocal, detailed reviews of these studies are not presented here.

The potential for elevated DE exposure in the occupational setting generally includes miners, railroad workers, truckers, bus and taxi drivers, heavy equipment operators, farm tractor drivers, and those involved with heavy duty marine engines. Regarding the mining industry some assert that excess lung cancer should be observed in the miners if exposure to DE is causally associated with the occurrence of lung cancer since DE is allegedly present in the mines. Our review of the mining industry data dose not support this assertion for the following reasons. In the United States, the introduction of diesel engines into metal mines dates from the early to the mid 1960s. Currently, there are approximately 265 underground metal/nonmetal mines in the United States. Virtually all of these mines use diesel powered equipment for various tasks, such as haulage, roof bolting etc. (Department of Labor, Mine Safety and Health Administration, 2001). Introduction of diesel equipment into coal mines was even later. Of 910 existing underground coal mines in the United States, only 145 currently use diesel-powered equipment. Of these 145 mines, 32 mines are currently using diesel equipment for face coal haulage. The remaining mines use diesel equipment for transportation, materials handling, and other support operations (Department of Labor, Mine Safety and Health Administration, 2001). It should be noted that there is a paucity of epidemiologic studies in miners where exposure to DE and health effects are explored. Furthermore, the majority of epidemiologic studies in miners do not mention exposure to diesel equipment use. Thus, it is impossible to know how many miners were exposed to DE and for how long and at what concentrations in a given study,

mode particles ranging up to 25-50 μm) and respiratory cancer for either sex (only respiratory symptoms and any-site female cancers were reported to be associated with TSP in this study).

The ACS Study and the later AHSMOG analyses (Abbey et al., 1995) also evaluated relationships between long-term exposures to sulfates (SO_4) (which are predominantly but not exclusively found in fine-mode particles, and can be considered an index for ambient fine particles) and lung cancer mortality. The ACS Study reported somewhat elevated and statistically significant lung cancer risk (RR = 1.36, 95% CI = 1.11-1.66) across 151 cities in combined analyses of data for both males and females. However, in further analyses of subgroups broken out by sex and smoking status (and thus having smaller sample sizes in each than for the above overall combined analyses), only the lung cancer mortality risks for male "ever-smokers" (RR = 1.44, 95% CI = 1.14-1.83) were statistically significant; no statistically significant relationships were reported for male "never-smokers" (RR = 1.36, 95% CI = 0.40 - 4.66), for female "ever-smokers" (RR = 1.10, 95% CI = 0.72-1.68), or for female "never-smokers" (RR = 1.61; 95% CI = 0.66 -3.92). In the later AHSMOG analyses, Abbey et al. (1995) found no statistically significant associations between sulfates and lung cancer or total mortality.

In summary, the three key prospective cohort studies summarized above, and discussed in more detail in the 1996 PM CD (U.S. EPA, 1996a), provide an equivocal array of results with regard to possible associations between chronic exposures to ambient PM and lung cancer mortality and/or incidence. None of the analyses of fine particles (as indexed by $PM_{2.5}$) in these three studies reported statistically significant relationships between long-term $PM_{2.5}$ concentrations and lung cancer mortality. Only the ACS Study found a statistically significant association of increased risk of lung cancer with one indicator of ambient fine particles (sulfates). Overall, then, these studies support a conclusion that there continues to be little epidemiologic evidence for an effect of ambient PM on lung cancer mortality or incidence. It is recognized, however, that subsequent AHSMOG analyses and other studies, published since completion of the 1996 PM CD, have further analyzed relationships between ambient PM and lung cancer. Results from these more recent studies are now being evaluated as part of the integrated assessment of ambient PM that will be part of the new PM CD targeted for completion in 2002.

7.2. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EXHAUST

An increased risk from malignancies of the lung, bladder, and lymphatic tissue has been reported in populations potentially exposed to higher levels of DE than typically seen in the environment. A few authors have reported other malignancies, including testicular cancer

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 DE exposure. From nearly 20,000 male employees in the early years, those aged 45 to 64 were followed for the 25-year period between 1950 and 1974 (the actual number of employees is not given in the paper), constituting a total of 420,700 man-years at risk. These workers were distributed among five job categories: drivers, garage engineers, conductors, motormen or guards, and engineers (works). Lung cancer were ascertained 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, and the results were combined 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 cancer 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%, motormen and guards had a mortality ratio of 87%, and both the bus drivers and conductors had mortality ratios of 75%. The engineers in the central works had a mortality ratio of 66%. These mortality ratios did not differ significantly from each other. Environmental sampling was done at one garage, on one day in 1979, for benzo[*a*]pyrene (B[*a*]P) concentrations and was compared with corresponding values recorded in 1957. Concentrations of B[*a*]P recorded in 1957 were at least 10 times greater than those measured in 1979.

This study failed to find any association between DE and occurrence of lung cancer, which may be due to several methodologic limitations. The lung cancer deaths were ascertained while the workers were 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 DE, and on lung cancer deaths among them, was not available for analysis. This 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. Therefore, there may not have been sufficient latency for the development of lung cancer. Use of greater London population death rates to obtain expected number of deaths may have resulted in a deficit in mortality ratios reflecting the "healthy worker effect." Investigators did not categorize the five

if any. Hence the studies in miners (coal, metal, and nonmetal with the exception of potash miners) are not reviewed in this chapter, because the available studies are uninformative relative to DE.

In this section, various mortality and morbidity studies of lung cancer from potential 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. In the United States the change from steam to diesel engines in locomotives began after World War II. By 1946 about 10% of the locomotives in service were diesel, by 1952 55% were diesel, and dieselization was about 95% complete by 1959 (Garshick et al., 1988). Therefore, exposure to DE was less common, and the follow-up period for studies conducted prior to 1960 (Raffle, 1957; Commins et al., 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 DE is greatly reduced; thus, they are not considered here.

On the other hand, the trucking industry changed to diesel trucks by the 1960s. In the 1960s sales of diesel-powered Class 8 trucks (long-haul trucks) were 48% of the market, and by the 1970s sales had risen to 85%. Thus, studies conducted among truck drivers prior to the 1970s may reflect exposures to gasoline exhaust as well as DE. Hence, studies with ambiguous exposures or studies that examined several occupational risk factors were excluded because they would have contributed little to the evaluation of the carcinogenicity of DE (Waxweiler et al., 1973; Williams et al., 1977; Ahlberg et al., 1981; Stern et al., 1981; Buiatti et al., 1985; Gustafsson et al., 1986; Siemiatycki et al., 1988). A study by Coggon et al. (1984) was excluded because occupational information abstracted from death certificates had not been validated; this would have resulted in limited information.

Several types of studies of the health effects of exposure to diesel engine emissions are reviewed in this chapter, such as cohort studies, case-control studies, and studies that conducted meta-analysis. In the cohort studies, cohorts of heavy construction equipment operators, railroad and locomotive workers, bus garage employees, and miners were studied retrospectively to determine increased mortality and morbidity resulting from exposures to varying levels of diesel emissions in the workplace. The evaluation of each study presents the study population, methodology used for the study, i.e., data collection and verification, analysis, results, and a critique of the study. There are some methodologic limitations that are common to studies with similar design. The total evidence, including limitations, is discussed at the end of the chapter in the summary and discussion section.

relative risks were calculated using the three exposure categories: nonexposed, possibly exposed, and probably exposed.

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 significantly elevated to 1.2 ($p=0.013$); and for those probably exposed, it was significantly elevated to 1.35 ($p=0.001$). 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 a risk of lung cancer of 1.00, 1.21, and 1.33 for those nonexposed, possibly exposed, and probably exposed to DE, 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 combustion fumes alone, retirement after 1950 shows the results of mixed exposure to coal combustion fumes and diesel fumes. As there was considerable overlap between occupations involving probable exposure to diesel fumes and probable exposure to coal, 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 combustion fumes 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 combustion fumes 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/coal combustion products and diesel fumes and not just one or the other. However, deaths due to lung cancer were not elevated among workers who retired prior to the 1950s and thus would have been primarily exposed to coal dust/coal combustion products. Furthermore, it should be noted that so far coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners. This

job categories either by qualitative or quantitative levels of DE exposure; neither 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 1974. It is not clear whether this age range was applied to calendar year 1950 or 1974, or at the midpoint of the 25-year follow-up period. No analyses were presented either by latency or by duration of employment (surrogate for exposure). The environmental survey based on B[*a*]P 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 B[*a*]P concentration occurred, because there are no environmental readings available between 1957 and 1979. It is also important to note that the concentrations of B[*a*]P 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. Thus, this study fails to provide either positive or negative association between the DE exposure and the occurrence of lung cancer.

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. The

The person-years of observation totaled 50,008 and were contributed by 8,490 individuals in the study, with a mean follow-up of 5.9 years. Only 2.2% (194) of the men were not traced. Observed deaths from all causes were significantly lower than expected (O = 495, $p<0.001$). 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 fewer than five) and merited cautious interpretation. Only a notable excess in the general hand category for lung cancer was based on as many as 48 cases (SMR = 133, $p<0.03$).

This mortality study did not demonstrate any cancer excess. Details of work history were not obtained to permit any analysis by DE exposure. The study's limitations, including small sample size, short duration of follow-up (average of only 6 years), and lack of sufficient latency period, make it inadequate to draw any conclusions.

7.2.1.4. *Wong et al. (1985): Mortality Among Members of a Heavy Construction Equipment Operators Union With Potential Exposure to DE Emissions*

This retrospective mortality study was conducted on a cohort of 34,156 male members of a heavy construction equipment operators union with potential exposure to DE emissions. Study cohort members were identified from records maintained at Operating Engineers' Local Union No. 3-3A in San Francisco, CA. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 year of membership in this union between January 1, 1964, and December 31, 1978, were included in the study. Work histories of the cohort were obtained from job dispatch computer tapes. The study follow-up period was 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

study was restricted to deaths among retired workers; therefore, it is unclear if a worker who developed lung cancer when actively employed and filed for a disability claim instead of retirement claim would be included in the study or not. Thus, it is possible that workers with heavy exposure might have been excluded from the study. Neither information on duration of employment in diesel work, nor coal dust-related jobs other than those held at retirement, nor details of how the exposure categories were created was provided. Therefore, it was not possible to evaluate whether this omission would have led to an under- or overestimate of the true relative risk. Although information on potential confounders such as smoking is lacking, the use of an internal comparison group to compute the relative risks minimizes the potential for confounding by smoking, as there is no reason to assume different smoking patterns among individuals exposed to DE versus those not exposed. Despite these limitations, this study provides suggestive evidence toward a causal association between exposure to DE and excess lung cancer.

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 (also known as rolling stock) garages and at Chiswick Works. The following information was obtained from computer listings: surname with initials, date of birth, date of joining company, last or present job, 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 residence was assumed to be close to their work; therefore place of work was coded as residence. One hundred different job titles were coded into 20 broader groups. These 20 groups were not ranked for DE 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. The workers 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.

worked on that job. Based on this classification system, if a person had ever worked in a high-exposure job title he was included in that group, even though he may have worked for a longer time in a low-exposure group or in an unknown exposure group. Information on length of work in any particular job, hence indirect information on potential length of exposure, was not available either.

For the high-exposure group a statistically significant excess was observed for cancer of the lung among bulldozer operators who had 15 to 19 years of membership and 20+ years of follow-up (SMR = 343, $p<0.05$). This excess was based on 5 out of 495 deaths observed in this group of 6,712 individuals, who contributed 80,328 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, O = 3, $p<0.05$) among those with 15 to 19 years of membership and length of follow-up of at least 20 years, and cancer of the liver (SMR = 872, O = 3, $p<0.05$) among those with 10 to 14 years of membership and length of follow-up of 10 to 19 years. There were 7,032 individuals who contributed to 78,403 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 = 174, O = 21, $p<0.05$). There were 3,656 individuals who contributed to 33,388 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 group comprised 9,707 individuals (28% of the cohort) contributing to 104,448 person-years. Statistically significant SMR excesses were observed for all cancers (SMR = 112, O = 339, $p<0.05$) and cancer of the lung (SMR = 119, O = 141, $p<0.01$). A significant SMR elevation was also observed for cancer of the stomach (SMR = 199, O = 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 actually resulted from metastases from the large intestine and/or rectum, as 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 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

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 15-year study period.

A total of 3,345 deaths was observed, compared with 4,109 expected. The corresponding SMR for all causes was 81 (p=0.01), which is consistent with the "healthy worker effect." A total of 817 deaths was attributed to malignant neoplasms, slightly fewer than the 878 expected based on U.S. white male cancer mortality rates (SMR = 93, p=0.05). Mostly there were SMR deficits for cause-specific cancers, including lung cancer for the entire cohort (SMR = 99, O = 309). The only significant excess SMR was observed for cancer of the liver (SMR = 167, O = 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 = 258). 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 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 (18,678 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 DE emissions), 20 functional job titles were used, which were further grouped into three potential categories: high exposure, low exposure, and unknown exposure. A person was classified in a job title if he ever

the 5-year age categories of person-years of observation to determine expected deaths for all causes, malignant diseases, and cardiovascular diseases. A Poisson distribution was used to calculate *p*-values and confidence limits for the ratio of observed to expected deaths. The total cohort of 694 men (after loss of 5 men to follow-up) was divided into three exposure categories: (1) clerks with 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 DE exposure, whereas seven were expected. The only other lung cancer death was observed among bus garage workers who had the highest DE exposure. This study's major limitations, including small size and poor data on DE exposure, make it inadequate to draw any conclusions.

7.2.1.6. *Boffetta and Stellman (1988): DE Exposure and Mortality Among Males in the American Cancer Society Prospective Study*

Boffetta and Stellman conducted a mortality analysis of 461,981 males with known smoking history and vital status at the end of the first 2 years of follow-up. The analysis was restricted to males aged 40 to 79 years in 1982 who enrolled in the American Cancer Society's prospective mortality study of cancer. Mortality was analyzed in relation to exposure to DE and to employment in selected occupations related to DE exposure. In 1982, more than 77,000 American Cancer Society volunteers enrolled more than 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

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 DE emissions was very common for this prolonged time, then the increase in lung cancer, as well as stomach cancer, might be linked to DE. 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 DE.

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, because 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 104,448 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. Fourth, 15 years' follow-up may not provide sufficient latency to observe excess lung cancer. 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.

7.2.1.5. *Edling et al. (1987): Mortality Among Personnel Exposed to DE*

This retrospective cohort mortality study of bus company employees investigated a possible increased mortality of cardiovascular diseases and cancers from DE 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

whereas 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 DE 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 main strength of this study is detailed information on smoking. The two main methodologic concerns 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 DE exposure. None of this information was validated. Nearly 20% of the individuals had an unknown exposure status to DE, and they experienced a higher mortality for all causes and lung cancer than both the DE exposed and unexposed groups. This could have introduced a substantial bias in the estimate of the association. Given that all DE 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. It should be noted that after adjusting for smoking, the RR reduced slightly from 1.41 to 1.31 and remained significant, indicating that observed excess of lung cancer was associated mainly with DE exposure.

7.2.1.7. *Garshick et al. (1988): A Retrospective Cohort Study of Lung Cancer and DE Exposure in Railroad Workers*

An earlier case-control study of lung cancer and DE 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 DE 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. DE 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 DE, 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, started work between 1939 and 1949 in the job codes sampled in 1959, and eligible for railroad benefits. To qualify for benefits, a worker must have had 10 years or more of service with the railroad 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 by a trained nosologist according to a system based on the ninth revision of the ICD.

The data were analyzed to determine the mortality for all causes and lung cancer in relation to DE exposure, mortality for all causes and lung cancer in relation to employment in selected occupations with high DE exposure, and mortality from other causes in relation to DE 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. Although data on 476,648 subjects comprising 939,817 person-years of risk were available for analysis, 3% of the subjects (14,667) had not given any smoking history, and 20% (98,026) did not give information on DE exposure and were therefore excluded from the main DE analysis. Among individuals who had provided DE exposure history, 62,800 were exposed and 307,143 were not exposed. Comparison of the population with known information on DE exposure with the excluded population with no information on DE 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.

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). The age-adjusted lung cancer relative risk was elevated significantly (RR = 1.41, 95% CI = 1.19,1.66), which was slightly decreased to 1.31 (95% CI = 1.10, 1.54). For lung cancer mortality the age- and smoking-adjusted 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 with 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 DE exposure was examined. For drivers who worked for 1 to 15 years, the relative risk was 0.87,

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 DE 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 DE in 1959 (n = 42,535), excluding 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 DE exposure in 1959. The results confirmed those obtained by using the proportional hazards model.

Relative risk estimates were then obtained using duration of DE 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 DE 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).

The results of this study, demonstrating a positive association between DE 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 (55,400) 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 DE-exposed, 94% of the cases were still in DE-exposed jobs 20 years later.

The main limitation of the study is the lack of quantitative data on exposure to DE in either individual workers or overall job categories. This is one of the few studies in which industrial hygiene measurements of DE 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 because exposed groups included individuals with low to high exposure. The number of years exposed to DE was used as a surrogate for dose. The dose, based on duration of employment, was inaccurate because

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, using the time dimension being the time since first entry into the cohort. The model also controlled for the birth year and the calendar time. The 95% confidence intervals were constructed using the asymptotic normality of the estimated regression coefficients of the proportional hazards model. Exposure was analyzed by DE-exposed jobs in 1959 and by cumulative number of years of DE exposure through 1980. Directly standardized rate ratios for deaths from lung cancer were calculated for DE 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 DE, 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

death ascertainment between 1977 and 1980 as incomplete. The Crump et al. (1991) analysis, limited to 1959 through 1976, found an excess lung cancer risk similar to the subsequent Garshick analysis (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991).

Garshick conducted some additional analyses after confirming the underascertainment of deaths by RRB identified by Crump et al. (1991). He reported that the relationship between years of exposure, when adjusted for attained age and calendar year, was flat to negative depending upon which model was used. He also found that in the years 1977-1980 the death ascertainment was incomplete; approximately 20% to 70% of deaths were missing depending upon the calendar year. Garshick's analysis, based on job titles in 1959 and limited to deaths occurring through 1976, showed that even though the relative risk for all exposure groups was elevated, the youngest workers still had the highest risk of dying of lung cancer.

Crump (1999), on the other hand, reported that the negative dose-response continued to be upheld in his latest analysis when age was controlled more carefully and years of exposure quantified more accurately. Crump (1999) asserted that the negative dose-response trends for lung cancer observed either with the cumulative exposure or with duration of exposure may be due to underascertainment of deaths in the last 4 years of follow-up of the Garshick et al. (1988) study as well as incomplete follow-up in earlier years.

California EPA's (Cal EPA, 1998) Office of Environmental Health Hazard Assessment (OEHHA) used the same railroad worker data for its quantitative risk assessment. The five job categories defined by Woskie et al. (1988a,b) and used by Crump et al. (1991) were combined into three exposure categories: exposed (engineers and firers; brakers, conductors, and hostlers; collectively known as "train workers"), unexposed (clerks and signalmen), and uncertain exposure (shop workers). In its analysis, OEHHA found a positive dose-response and a steadily increasing risk of lung cancer with increasing duration of exposure by using age in 1959 but allowing for an interaction term of age and calendar year in the model. This positive dose-response finding was contradictory to the negative to flat dose-response findings of both Crump et al. (1991) and Garshick (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991).

The Health Effects Institute (HEI, 1999) convened an expert panel specifically to evaluate strengths and limitations of two epidemiologic studies that had some exposure data, for quantitative risk estimation and to resolve the discrepancies in the dose-response results reported by Garshick et al. (1988), Crump et al. (1991), and OEHHA (Cal EPA, 1998). In their evaluation of the epidemiologic study of railroad worker data for quantitative risk assessment, the panel conducted their own analysis of the Garshick et al. (1988) data. They excluded the last 4 years of follow-up (1977-1980) because of underascertainment of deaths during these years.

individuals were working on steam and diesel locomotives during the transition period. It should be noted that the investigators only included exposures after 1959; the duration of exposure prior to 1959 was not known. If the categories of exposure to DE had been set up as no, low, moderate, and high exposure, the results would have been more meaningful, as would the dose-response relationship. Another limitation of this study was its inability to examine the effect of years of exposure prior to 1959 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 indicate that occupational exposure to DE is associated with a modest risk (1.5) of lung cancer.

The data of this study were used by Crump et al. (1991) to explore the development of dose-response-based quantitative estimates of lung cancer associated with DE exposure by using diesel exposure estimate data from the industrial hygiene (IH) studies conducted by Hammond (1998) and Woskie et al. (1988a,b). These studies were conducted in conjunction with the Garshick et al. (1988) study. The Woskie et al. (1988a,b) IH studies were conducted in four small northern railroads where the workers were exposed to DE in the early 1980s, prior to the Garshick et al. (1988) epidemiologic study. A total of 39 job titles were identified by Woskie et al. (1988a,b), which were subsequently combined into 13 job groups and finally merged into 5 career exposure job codes as follows: brakers, conductors, and hostlers; clerks; engineers and firers; signal maintainers; and shop workers. The average exposure estimates were assigned to the cohort members by Crump et al. (1991) based on the job codes in 1959. Cumulative exposures were calculated using these average exposures for each job code. The exposures in the IH study by Hammond (1998) were defined as the concentrations of respirable-sized particles (RSP), the adjusted respirable particles (ARP) concentrations, and the adjusted extractable mass (AEM). The concentrations of ARP were estimated in the IH study by removing the particle contribution of environmental tobacco smoke (ETS). Crump et al. (1991) also used another index called total extractable material (TEX), which was the extractable RSP including the particle contribution of ETS. Using these four exposure indices and the regional climates for the United States, Crump et al. (1991) constructed various exposure metrics. They conducted more than 50 analyses based on calendar year, age in 1959, attained age, and five job codes identified in 1959: brakers, conductors, and hostlers; clerks; engineers and firers; signal maintainers; and shop workers; using the exposure metrics. Crump et al. (1991) used the U.S. general population age- and year-specific death rates for comparison and found that the relative risk can be positively or negatively related to the duration of exposure depending on how age was controlled in a model. Their use of the U.S. general population rates instead of the internal unexposed group of railroad workers that was used by Garshick et al. (1988) identified that the

duration of employment, the lack of a positive exposure-response association in the railroad worker cohort substantially weakens that study's potential to provide a reliable quantitative estimate of risk of exposure to diesel engine emissions." Thus, the panel recommended against using the current railroad worker data as the basis for quantitative risk assessment in ambient settings.

The panel also reported that the Garshick et al. study (1987, 1988) had several strengths, such as a large number of study subjects (55,407 subjects, including 1,694 lung cancer deaths in the cohort study and 1,256 lung cancer cases for the case-control study). The workers were employed in an industry where many of them were exposed to DE. Confounding by asbestos was handled by either excluding certain job categories from the analyses or controlling for it in the analyses. Confounding by smoking was controlled in the analyses of case-control study. The panel concluded that the overall results of the Garshick studies were generally consistent with findings of a weak association between exposure to DE and occurrence of lung cancer.

Thus, it should be noted that although the railroad worker data are unsuitable for quantitative risk assessment, they provide qualitative support for a positive association between exposure to DE and occurrence of lung cancer.

7.2.1.8. *Gustavsson et al. (1990): Lung Cancer and Exposure to DE Among Bus Garage Workers*

A retrospective mortality study (from 1952 to 1986), cancer incidence study (from 1958 to 1984), and nested case-control study were conducted among a cohort of 708 male workers from five bus garages in Stockholm, Sweden, who had worked for at least 6 months between 1945 and 1970. Thirteen individuals were lost to follow-up, reducing the cohort to 695.

Information was available on location of workplace, job type, and beginning and ending of work periods. Workers were traced through 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. Six controls matched on age ± 2 years, selected from the noncases at the time of the diagnosis of cases, were drawn at

The panel categorized the duration of exposure in 12 categories that were basically the duration of employment. The exposure was assumed to be linearly increasing for 15 years prior to 1959. Lags of 5 and 10 years were also considered in the analysis. The job categories based on job held in 1959 were classified as clerks, signalmen, engineers and firers, conductors and brakers, hostlers, and shop workers. For final analysis these were collapsed into three groups: clerks and signalmen, train workers (engineers and firers, conductors and brakers, and hostlers), and shop workers. Seven different models were used. The panel's analysis revealed consistently elevated lung cancer risk for train workers compared with clerks for each duration of employment (1-4, 5-9, 10-14, 15-17, 18+) in years and that shop workers had an intermediate risk of lung cancer. Their analysis also revealed decreasing risk of lung cancer with increasing duration of employment in all three job categories. These findings were similar to those of Garshick (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991) and Crump et al. (1991).

In addition to differences in adjusting the age (age in 1959 versus attained age) in their respective analyses, these three investigators made different assumptions in estimating exposure patterns in these railroad workers. Garshick et al. (1988) assumed that there was no exposure to DE prior to 1959 and that the exposure to DE was constant throughout the period of follow-up, i. e., 1959 to 1980 (block exposure pattern). Crump et al. (1991) assumed that the exposure to DE increased steadily from 1945 to 1959 to the same level as assumed in the block exposure pattern by Garshick et al. (1988) and then remained constant from 1959 through 1980 (ramp exposure pattern). OEHHA assumed that the exposure increased steeply from 1945 to 1959. The peak exposure attained in 1959 according to OEHHA was twice as high as assumed in the block and ramp exposure patterns by Garshick et al. (1988) and Crump et al. (1991), respectively. The exposures then declined steeply from 1959 to reach the levels assumed in the block and ramp exposure patterns in 1980 (roof exposure pattern). The roof exposure pattern was constructed on the assumption that diesel engines were "smokier" in the past. A detailed discussion of divergent results observed by Crump and Cal EPA can be found in Chapter 8.

The panel discussed various possibilities for the negative dose-response found among train workers and to a lesser extent among shop workers. They asserted that several types of biases could affect the data, alone or in combination, and mask a true positive association. The biases enumerated by the panel were: unmeasured confounding by smoking, exposure to other sources of pollution, previous occupational exposures, exposure misclassification, use of "duration of employment" as a surrogate measure for exposure, healthy worker survivor effect, and differential or incomplete ascertainment of lung cancer deaths (for detailed discussion of how an individual bias affects the results, please see HEI, 1999). The panel concluded, "However, despite the reason or reasons why the relative risks in these data decrease with

unity, because exposure classification was done independently of the outcome. Although the analysis by dose indices was done, no latency analysis was performed. Although data on smoking were missing, it is unlikely to confound the results because this is a nested case-control study; therefore, smoking is not likely to be different among the individuals irrespective of their exposure status to DE. Overall, this study provides some support to the excess lung cancer results found earlier among populations exposed to DE.

7.2.1.9. *Hansen (1993): A Follow-up Study on the Mortality of Truck Drivers*

This is a retrospective cohort mortality study of unskilled male laborers, ages 15 to 74 years, in Denmark, identified from a nationwide census file of November 9, 1970. The exposed group included all truck drivers employed in the road delivery or long-haul business (14,225). The unexposed group included all laborers in certain selected occupational groups considered to be unexposed to fossil fuel combustion products and to resemble truck drivers in terms of work-related physical demands and various personal background characteristics (43,024).

Through automatic record linkage between the 1970 census register (the Central Population Register 1970 to 1980) and the Death Certificate Register (1970 to 1980), the population was followed for cause-specific mortality or emigration up to November 9, 1980. Expected number of deaths among truck drivers was calculated by using the 5-year age group and 5-year time period death rates of the unexposed group and applying them to the person-years accumulated by truck drivers. 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 was due mainly 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 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, O = 19, 95% CI = 138 to 358) and 60 to 64 (SMR = 227, O = 22, 95% CI = 142 to 344) age groups only.

As acknowledged by the author, the study has quite a few methodologic limitations. The exposure to DE is assumed in truck drivers based on use of diesel-powered trucks, but no validation of qualitative or quantitative exposure is attempted. It is also not known whether any

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.

DE and asbestos exposure assessments were performed by industrial hygienists based on the intensity of exposure to DE and asbestos, specific for workplace, work task, and calendar time period. A DE 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 DE 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 contribution from all work periods for both DE and asbestos. Four DE 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, SMRs for lung cancer were 122 and 115 using Stockholm occupationally active and general population, respectively. No dose-response was observed with increasing cumulative exposure in the mortality study. The cancer incidence study reportedly confirmed the mortality results (results not given).

The nested case-control study, on the other hand, showed increasing risk of lung cancer with increasing exposure. Using 0 to 10 DE exposure index as the comparison group yielded 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 DE indices 10 to 20, 20 to 30, and >30, respectively. The study was based on 17 cases and 6 controls for each case matched on age ± 2 years. Adjustment for asbestos exposure did not change the lung cancer risk for DE.

The main strength of this study is the detailed exposure matrices constructed for both DE 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

exposures and were expressed in intervals of 0.5 ymg/m^3. Both the exposure data and the smoking data obtained from the medical files were validated by personal interviews with 1,702 cohort members. Death certificates were obtained from local health centers for 94.4% of deceased members. Autopsy data were available for 13% of the deceased. Internal comparison was done between production and workshop categories. Using East German general male population rates, SMRs were computed for the total cohort as well as the subcohort. Analyses were done using Poisson and Cox regression models.

The concentrations of total carbon for production, maintenance, and workshop categories were 0.39 mg/m^3, 0.23 mg/m^3, and 0.12 mg/m^3, respectively. The cumulative exposure ranged from 0.25 ymg/m^3 to 6.25 ymg/m^3. The regression analysis showed that the cohort's smoking habits were homogenous and that smoking had an even distribution over cumulative exposure.

A total of 424 deaths were observed for the entire cohort (SMR = 54). The all-cancer deaths were 133, of which 38 were from lung cancer (SMR = 78). Analysis for the subcohort using the internal comparison group of low exposure (workshop category, mean cumulative exposure = 2.12 ymg/m^3) RR of 2.17 (95% CI = 0.79, 5.99) was found for the production category (mean cumulative exposure = 4.38 ymg/m^3). The relative risks for lung cancer for 20 years of exposure in the production category (highest exposure = cumulative exposure of 4.9 ymg/m^3) were calculated using Poisson and Cox regression methods. RRs of 1.16 and 1.68 were observed for the total cohort, while RRs of 1.89 and 2.7 were observed for the subcohort by Poisson and Cox regression methods respectively.

The main strengths of the study are the information available on DE exposure and smoking. Although these potash miners were exposed to salt dust and nitric gases, exposures to other confounders such as heavy metals and radon were absent. Smoking does not seem to be a confounder in this study but cannot be completely ruled out. Unfortunately, the age distribution of the cohort is not available. Since there were only 424 deaths in 25 years of follow-up in this cohort of 5,536, it appears that the cohort is young. Although lung cancer risk was elevated by twofold in the production category of the subcohort of miners who had worked for at least 10 years underground at the same job for 80% of their time and did not have more than 3 jobs, it was not statistically significant. The follow-up period for this study was 25 years, but the cohort members could have entered the cohort any time between 1970 and 1990, as long as they worked underground for a year, i.e., they could have worked in the mines for 1 year to 21 years. Thus, the authors may not have had enough follow-up or latency to observe the lung cancer excess. Despite these limitations, the results of this study provide suggestive evidence for the causal association between DE and excess lung cancer.

Table 7-1 summarizes the above cohort studies.

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 DE. The truck drivers and the unexposed laborers were from the same socioeconomic class and may have the same smoking habits. Still, the lack of information on smoking data and a 36% rural population (usually consuming less tobacco) in the unexposed group may potentially confound the lung cancer results. However, a population survey carried out in 1988 showed very little difference in smoking habits of residents of rural areas and the total Danish male population. The investigator reports that diesel trucks were introduced in Denmark after World War II, and since the late 1940s the majority of the Danish fleet has been composed of diesel trucks. Consequently, even though the follow-up period is relatively short, the truck drivers may have had exposure to DE for 20 to 30 years. Therefore, the finding of excess lung cancer in this study is consistent with the findings of other truck driver studies.

7.2.1.10. *Saverin et al. (1999): DE and Lung Cancer Mortality in Potash Mining*

This is a cohort mortality study conducted in male potash miners in Germany. The mines began using mobile diesel-powered vehicles in 1969 and 1970. Miners who had worked underground for at least 1 year after 1969 to 1991, when the mines were closed, were followed from 1970 to 1994. A total of 5,981 individuals were identified from the medical records by a team of medical personnel familiar with the mining technology. A total of 5,536 were eligible for follow-up after 5.5% were excluded due to implausible or incomplete work history and 1.9% were lost to follow-up. A subcohort of 3,258 miners who had worked for at least 10 years underground (80% had held a single job) was also identified. The miners' biannual medical examination records were used to extract the information about personal data, smoking data, and pre-mining occupation, and to reconstruct a chronology of workplaces occupied by the worker since hire for each person.

Exposure categories were defined as production, maintenance, and workshop, roughly corresponding to high, medium, and low. Concentrations of total carbon, including elemental and organics, were measured in the airborne fine dust in 1992. A total of 255 samples covering all workplaces was obtained. Most were personal dust samples; some were area dust samples. Cumulative exposure was calculated for each miner, for each year of observation, using the work chronology and the work category. For the workshop category years of employment were considered as exposure time; for production and maintenance years of employment was weighted by a factor of 5/8, since these workers for an 8-hour shift worked for only 5 hours underground. As neither the mining technology nor the type of machinery used had changed substantially from 1970 to 1992, the exposure measurements were considered to represent the exposures throughout the study period. Accrued person-years were classified into cumulative

Table 7-1. Epidemiologic studies of the health effects of exposure to DE: cohort mortality studies (continued)

Authors	Population studied	DE exposure assessment	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 DE 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 DE exposure without any ranking Average 6-year follow-up i.e., not enough time for lung cancer latency No adjustment for confounders
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 DE emissions	SMR = 166 (p<0.05) for liver cancer for total cohort SMR = 343 (observed = 5, p<0.05) for lung cancer for high-exposure bulldozer operators with 15-19 years of membership, 20+ years of follow-up SMR = 119 (observed = 141, p<0.01) for workers with no work histories	No validation of exposure categories, which were based on surrogate information Incomplete employment records Employment history other than from the union not available 15 year follow-up may not provide sufficient time for lung cancer latency No data on confounders such as other exposures, alcohol, smoking, etc.
Edling et al. (1987)	694 male bus garage employees Follow-up from 1951 through 1983 Mortality of these men was compared with mortality of general population of Sweden	Three exposure groups based on job titles: High exposure, bus garage workers Intermediate exposure, bus drivers Low exposure, clerks	No SS differences were observed between observed and expected for any cancers by different exposure groups	Small sample size No validation of exposure No data on confounders such as other exposures, smoking, etc.

Table 7-1. Epidemiologic studies of the health effects of exposure to DE: cohort mortality studies

Authors	Population studied	DE exposure assessment	Results	Limitations
Waller (1981)	Approximately 20,000 male London transportation workers Aged 45 to 64 years 25 years follow-up (1950-1974)	Five job categories used to define exposure Environmental B[*a*]P 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 B[*a*]P 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/combustion products and DE No validation of method was used to categorize exposure Lack of data on smoking but use of internal comparison group to compute RRs minimizes the potential confounding by smoking No latency analysis

Table 7-1. Epidemiologic studies of the health effects of exposure to DE: cohort mortality studies (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
Garshick et al. (1988)	55,407 white male railroad workers Aged 40 to 64 years in 1959 Started work 10-20 years earlier than 1959	Industrial hygiene data correlated with job titles to dichotomize the jobs as "exposed" or "not exposed"	RR = 1.45 (40-44 year age group) RR = 1.33 (45-49 year age group) Both SS After exclusion of workers exposed to asbestos RR = 1.57 (40-44 year age group) RR = 1.34 (45-49 year age group) Both SS	Years of exposure used as surrogate for dose Not possible to separate the effect of time since first exposure and duration of exposure Lack of smoking data but case-control study showed very little difference between those exposed to DE versus those who were not
Garshick (ltr to Chao Chen, EPA, dtd 8/15/91			Dose response indicated by increasing lung cancer risk with increasing cumulative exposure Further analysis using attained age, limited through 1976 showed youngest workers still had the highest risk	
Crump et al. (1991) Crump (1999)	Reanalysis of Garshick et al., 1988 data		Dose response found to be positive or negative depending upon how the age was controlled in the model Negative dose-response upheld in the latest analysis	
California EPA (1998)	Reanalysis of Garshick et al., 1988		Positive dose response using age at 1959 and interaction term of age & calendar year	

Table 7-1. Epidemiologic studies of the health effects of exposure to DE: cohort mortality studies (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
Boffetta and Stellman (1988)	46,981 male volunteers enrolled in the American Cancer Society's Prospective Mortality Study of Cancer in 1982 Aged 40 to 79 years at enrollment First 2-year follow-up	Self-reported occupations were coded into 70 job categories Employment in high DE exposure jobs were compared with nonexposed jobs	Total mortality (SS) elevated for railroad workers (RR=1.43), heavy equipment operators (RR=1.7), miners (RR=1.34), and truck drivers (RR=1.19) Lung cancer mortality (SS) adjusted for age & smoking, elevated for total cohort (RR=1.31), miners (RR=2.67), and heavy equipment operators (RR=2.6) Lung cancer mortality (SNS) elevated among railroad workers and truck drivers Truck drivers also showed a dose-response	Exposure information based on self-reported occupation for which no validation was done Volunteer population, probably healthy population

7.2.2. Case-Control Studies of Lung Cancer

7.2.2.1. *Hall and Wynder (1984): A Case-Control Study of DE 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 DE exposure and lung cancer. Histologically confirmed primary lung cancer patients who were 20 to 80 years old were ascertained from 18 participating hospitals in 6 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 DE 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 DE 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 DE 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 DE versus those who were not. The risk, however, decreased to a nonsignificant 1.4 when the data were adjusted for smoking.

Table 7-1. Epidemiologic studies of the health effects of exposure to DE: cohort mortality studies (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
Gustavsson et al. (1990)	695 male workers from 5 bus garages in Stockholm, Sweden, who had worked for 6 months between 1945 and 1970 34 years follow-up (1952-1986) Nested case-control study 17 cases, six controls for each case matched on age ± 2 years	Four DE indices were created: 0 to 10, 10 to 20, 20-30, and >30 based on job tasks and duration of work	SNS SMRs of 122 and 115 (OA and GP), respectively Case-control study results showed dose response: 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 is unlikely to confound the results since it is a nested case-control study
Hansen (1993)	Cohort of 57,249 unskilled laborers, ages 15 to 74, in Denmark (nationwide census file) November 9, 1970 Follow-up through November 9, 1980	DE exposure assumed based on diesel-powered trucks	SS SMRs for lung cancer : SMR = 160 for total population SMR = 229 for age 55-59 years SMR = 227 for age 60-64 years	No actual exposure data available Lack of smoking data but population survey showed very little difference between rural and urban smoking habits Job changes may have occurred from laborer to driver Short follow-up period
Saverin et al. (1999)	Cohort of 5,536 potash miners who had worked underground for at least 1 year after 1969 Subcohort of 3,258 who had worked for at least 10 years underground Follow-up from 1970 to 1994	DE exposure categories defined as: production (high) maintenance (medium) workshop (low) 225 air samples obtained: for total carbon, organics, & fine dust in 1992	SNS increased RRs adjusted for smoking: 1.68 and 2.7 for total cohort & subcohort, respectively	Small, young cohort Few deaths No latency analysis

Abbreviations: RR = relative risk; SMR = standardized mortality ratio; SNS = statistically nonsignificant; SS = statistically significant; O = occupationally active; GP = general population.

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 = 0.9, 2.6) and 9.8 (95% CI = 1.5, 414). These were the only two occupations listed with potential DE exposure. An excess significant risk was detected for copper smelter workers, plumbers, electricians, and asbestos workers, as well as concrete and asphalt workers. All the odds ratios were calculated by adjusting for age, smoking, and municipality. A comparison with the live controls resulted in the odds ratios being lower than those observed with dead controls, and none 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, DE, 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 results in either direction.

7.2.2.3. *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

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 DE 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 DE 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 DE exposures. Furthermore, the exposure categories based on job titles were broad, and thus would have made a true effect of DE difficult to detect.

7.2.2.2. *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-cell 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%).

least five controls for only two industries, construction and painting, for which the OR 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 two ethnic groups, southwestern Hispanic and non-Hispanic white males. The major limitations pertain to the occupational exposure data. 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 DE 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 DE 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 DE exposure.

7.2.2.4. *Garshick et al. (1987): A Case-Control Study of Lung Cancer and DE 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 workers exposed to DE compared with those who were not. Based on 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 selected randomly from workers

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 high-risk jobs for lung cancer, the principal investigator created an a priori listing of suspected occupations and industries by a two-step process involving a literature review for implicated industries and occupations. The principal investigator also determined the appropriate Standard Industrial Classification and Standard Occupational Codes associated with job titles. For four agents—asbestos, wood dust, DE, 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 DE 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 (OR) adjusted for age, smoking, and ethnicity was not elevated for DE fumes (OR = 0.6, 95% CI = 0.2, 1.6). Significantly elevated ORs were found for uranium miners (OR = 2.8), underground miners (OR = 2.4), construction workers, and welders (OR = 4.3). No excess risks were detected for the following industries: shipbuilding, petroleum refining, 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

Increasing years of DE 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 DE exposure, the odds ratio was 1.64 (95% CI = 1.18, 2.29), whereas among individuals who had 5 to 19 years of DE 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 DE 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.

Alternative models to explain past 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 DE 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 DE 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 OR were not significant.

The many strengths of the study are consideration of confounding factors such as asbestos exposure and smoking; classification of DE exposures by job titles and industrial hygiene sampling; exploration of interactions between smoking, asbestos exposure, and DE 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 DE exposure jobs with low intensity and intermittent exposure, such as railroad police and bus drivers, as unexposed. These two limitations would result in underestimation of the lung cancer risk. This source of error could have been avoided if DE 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

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 DE 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 DE 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 DE 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 DE exposure. Those jobs for which no personal sampling was done were considered exposed or unexposed based on similarities in job activities and work locations and by degree of contact with diesel equipment. Asbestos exposure was categorized based on 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 DE, whereas among older workers, only 47% were exposed to DE.

Analysis by a regression model, in which years of DE 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 >20 years of DE 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.

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.

7.2.2.6. *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

occupational or environmental exposures and passive smoking. In conclusion, this study provides strong evidence that occupational DE emission exposure increases the risk of lung cancer.

7.2.2.5. *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, and 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 RR 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.

7.2.2.7. *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 DE 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). ORs by duration of employment as a categorical variable were then estimated. For the Teamsters Union work history data, 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

exposure to known and suspected lung carcinogens. ORs 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. ORs 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. ORs 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 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 statistically 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 ORs 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 carcinogens. 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 DE or gasoline exhaust or a mixture of the two. Among the study's other limitations are a possible bias due to misclassification of jobs reported by the large proportion of next-of-kin interviews. Such a bias would make the effect of DE harder to detect due to broad categorization of jobs 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.

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. ORs 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. ORs 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 were found to have the highest exposures and relative risk, followed by long-haul and then short-haul drivers, although dockworkers had 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 DE 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 terms 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 ORs 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 survey found that mechanics had the highest current levels of DE exposures and dockworkers who mainly used propane-powered forklifts had the lowest exposure. ORs of 1.69

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). Similarly, the corresponding odds ratio was 1.34 (95% CI = 0.81, 2.22) for both gasoline truck drivers and drivers who drove both types of trucks, and 1.09 (95% CI = 0.44, 2.66) for truck mechanics.

No significant interactions between age and DE exposure or smoking and DE exposure were observed. All the ORs were adjusted for age, smoking, and asbestos in addition to various exposure categories.

This is a well-designed and analyzed study. The main strengths of the study are the availability of detailed records from the Teamsters Union, a relatively large sample size, availability of smoking data , and measurements of exposures. The authors acknowledge some 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, they could not evaluate the concordance between Teamsters Union and next-of-kin job categories easily because job categories were defined differently in each data set. No data were available on levels of diesel exposure for the different job categories. Despite these limitations, the positive findings of this study, which are probably underestimated, provide a positive evidence toward causal association between DE exposure and excess lung cancer.

7.2.2.8. *Steenland et al. (1998): DE 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 exposure, a surrogate for DE 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

the use of these data for quantitative risk assessment, due to limitations of the exposure data. As far as qualitative risk assessment is concerned, this study is still considered to be positive and strong.

7.2.2.9. *Boffetta et al. (1990): Case-Control Study on Occupational Exposure to DE 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 DE at the workplace or during hobby activities. A priori aggregation of occupations was categorized into low probability of DE 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 DE 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 DE exposure group and the subset of post-1985 enrollees for highest duration of exposure (for self-reported exposure, occupations with probable exposure, and truck drivers), none was statistically significant. Trend tests for the risk of lung cancer among self-reported DE 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), because it

and 0.93 were observed for the mechanics and dockworkers, respectively. The finding of the highest lung cancer risk for mechanics and lowest for dockworkers is indicative of causal association between the DE exposure and development of lung cancer. The log of cumulative exposure was found to be the best-fitting model and was a significant predictor ($p = 0.01$). However, the risk among mechanics did not increase with increasing duration of employment.

OR 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 found that current exposures indicated that truck drivers are exposed to DE at levels about the same as ambient levels on the highways, which are about double the background levels in urban air. They conclude that the data suggest a positive and significant increase in lung cancer risk with increasing estimated cumulative exposure to DE 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 the authors 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 DE 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 concerns about the exposure estimations, selection of controls, and control for confounding variables, and hence, this study's usefulness for quantitative risk assessment. EPA and NIOSH will address these concerns in the year 2001. The HEI (1999) panel noted that some of the strengths of this study include the relevance of exposure levels to the general population and the use of an exposure marker for diesel engine emissions that was an improvement over the concentration of respirable-size particles (RSP). The number of study subjects (996 lung cancer cases) is large. Histories of exposures to asbestos and smoking were obtained, and confounding by these two variables was controlled in the analysis. Thus, it should be noted that these concerns are about

"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 DE 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 DE 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 DE and smoking. However, with just six nonsmokers and no further categorization of smoking amount or duration, these results are of limited value.

The DE exposure matrices created using three different variables are intricate. Analyses by any of these variables yield essentially the same positive results and positive trends, providing consistent support for a real effect of DE 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 DE 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 DE.

was difficult to estimate or verify the actual exposure to DE, duration of employment was used as a surrogate for dose instead. The numbers of cases and controls were large; however, the number of individuals exposed to DE was relatively few, thus reducing the power of the study. This study did not attempt latency analysis either. Due to these limitations, the findings of this study are unable to provide either positive or negative evidence for a causal association between DE and occurrence of lung cancer.

7.2.2.10. *Emmelin et al. (1993): DE Exposure and Smoking: A Case-Referent Study of Lung Cancer Among Swedish Dock Workers*

This case-control study of lung cancer was drawn from a 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 DE 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 DE 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,

among males born between 1897 and 1966 were identified from the Danish Cancer Registry. The registry provided the information on diagnosis from ICD-7, name, sex, and unique personal identification number (PIDN). Information about past employment was obtained by linkage with the nationwide pension fund. The fund keeps the records by name and PIDN about the date of start and end of each job and unique company number of the employer. The records are kept even after the employee has retired or died. Information about current employment was obtained from the Danish Central Population Registry (CPR) by linkage with the PIDN.

Of 37,597 cases identified from the Registry, 8,853 did not have any employment records. Controls (1:1) for 28,744 lung cancer cases with employment histories were selected randomly from CPR, matched with the case by year of birth and sex. Furthermore, these controls had to be alive, cancer free, and employed prior to the diagnosis of lung cancer in the corresponding case. Employment histories were obtained for the controls in the same fashion as cases from the pension fund. The employment record search resulted in a total of 1,640 lorry/bus drivers and 426 taxi drivers. They were further divided into subgroups by their duration of employment. Information about smoking in drivers was acquired from two national surveys conducted in 1970-72 and 1983. No direct information on smoking was available in either cases or controls. A separate case-control study of mesothelioma indirectly looked at asbestos exposure among professional drivers. OR, adjusting for socioeconomic status and 95% CI, were computed using conditional logistic regression (PECAN procedure in the statistical package EPICURE).

Significant ORs for lung cancer were found for lorry/bus drivers (OR = 1.31, 95% CI = 1.17, 1.46), taxi drivers (OR = 1.64, 95% CI = 1.22, 2.19), and unspecified drivers (OR = 1.39, 95% CI = 1.30, 1.51). Significant ORs were found for both lorry/bus drivers and taxi drivers by duration of employment in 1-5 years and >5 years categories, with no lag time and with a 10-year lag time. The ORs remained the same for lorry/bus drivers in these employment categories for no lag time and 10-year lag time. Among taxi drivers, on the other hand, the OR of 2.2 in >5 year employment in no-lag-time analysis increased to 3.0 in the 10-year lag time analysis. The authors asserted that the higher risk seen in the taxi drivers may be due to higher exposure attributable due to longer time spent in traffic congestion. The trend tests for increasing risk with increasing duration of employment (surrogate for exposure) were statistically significant ($p<0.001$) for both lorry/bus drivers and taxi drivers in no-lag-time and 10-year lag time analysis. All the ORs were adjusted for socioeconomic status.

The main strengths of the study are the large sample size, availability of information on socioeconomic status, and detailed employment records. The main limitation, however, is lack of information on what type of fuel these vehicles used. It is probably safe to assume that the lorry/buses were diesel powered, whereas the taxis could be either diesel or gasoline powered. A

7.2.2.11. *Swanson et al. (1993): Diversity in the Association Between Occupation and Lung Cancer Among Black and White Men*

This population-based case-control study of lung cancer was conducted in metropolitan Detroit. The cases and controls for this study were identified from the Occupational Cancer Incidence Surveillance Study (OCISS). A total of 3,792 incident lung cancer cases and 1,966 colon and rectal cancer cases used as controls, diagnosed between 1984 and 1987 among white and black males aged 40 to 84 years, were selected for the study. Information was obtained by telephone interview either with the individual or a surrogate about lifetime work history and smoking history, as well as medical, demographic, and residential history. Occupation and industry data were coded using the 1980 U.S. Census Bureau classification codes. The investigators selected certain occupations and industries as having little or no exposure to carcinogens and defined them as an unexposed group. Analysis was done using logistic regression method and adjusting for age at diagnosis, pack-years of cigarette smoking, and race.

The results were presented by various occupations and industries; those with potential exposures to DE were drivers of heavy trucks and light trucks, farmers, and railroad workers, respectively. Among white males, increasing lung cancer risks were observed with increasing duration of employment for drivers of heavy trucks, drivers of light trucks, and farmers. Although none of the individual ORs were statistically significant, trend tests were significant for all three occupations ($p \leq 0.05$). On the other hand, among black males increasing lung cancer risks with increasing duration of employment were observed for farmers only, with an OR of 10.4 (95% CI = 1.4, 77.1) reaching significance for employment of 20+ years. As for the railroad industry, increasing lung cancer risks with increasing duration of employment were observed for both white and black males. The trend test was significant for white males only, with an OR of 2.4 (95% CI = 1.1, 5.1) reaching significance for employment of 10+ years.

The main strengths of the study are large sample size, availability of lifetime work history and smoking history, and the population-based study format, precluding selection bias. The major limitation, as in other studies, is lack of direct information on specific exposures. The interesting result of this study is lung cancer excesses observed in farmers, mainly among crop farmers, who have potential exposure to DE from their tractors in addition to pesticides, herbicides, and other PM_{10}. The authors point out that this is the first study to find excess lung cancer in this occupation.

7.2.2.12. *Hansen et al. (1998): Increased Risk of Lung Cancer Among Different Types of Professional Drivers in Denmark*

This is a population-based case-control study of lung cancer, conducted in professional drivers in Denmark. The cases first diagnosed as primary lung cancer between 1970 and 1989

with DME exposure. The exposure assessment was done without knowing the status of the case/control.

For each individual, cumulative exposure was calculated for the complete work history by categorizing the duration of exposure as >0-3, >3-10, >10-20, >20-30, >30 years, and beginning and end of exposure. The first year of exposure was defined as ≤1945, 1946-1955, and ≥1956 while the last year of exposure was defined as ≤1965, 1966-1975, and ≥1976. For professional drivers, hours driven per day were accumulated and were classified as "driving hours."

A smoker was defined as any individual who had smoked regularly for at least 6 months. Smoking information was acquired in series with the starting time, type of tobacco, amount smoked, duration in years, and calender year of quitting. Asbestos exposure was estimated by certain job-specific supplementary questions.

The cases and controls were post-hoc stratified into 6 age and 17 region categories. ORs adjusted for smoking and asbestos exposure were calculated by conditional logistic regression, using "never exposed" workers as the reference group. The adjustment for cigarette smoking was done by using pack-years as a continuous variable; adjustment for other tobacco products was done by considering them as a binary variable. A total of 716 cases and 430 controls were found to be ever exposed to DME. The smoking- and asbestos-adjusted OR of 1.43 (95% CI = 1.23, 1.67) for all DME exposed was reduced from the crude OR of 1.91. For the entire group the various analyses yielded statistically significant ORs ranging from 1.25 to 2.31, adjusted for smoking and asbestos exposure (West Germany, >10-20 years and >20-30 years of exposure, first year of exposure in 1946-1955 and 1956+, end of exposure in 1966-1975 and 1976+, and for the job categories of Group A, B, and C). The risk increased with increasing years of exposure, and for both the first year of exposure (≤1945, 1946-1955, and ≥1956) and end year of exposure (≤1965, 1966-1975, and ≥1976).

Separate analyses by four job categories (all the ORs were adjusted for smoking and asbestos exposure) showed that for professional drivers (Group A) the overall OR was 1.25 (95% CI = 1.05, 1.47). Significant ORs were found for various factors in West Germany only. The factors were: >0-3 years and >10-20 years of exposure (OR = 1.69, 95% CI = 1.13, 2.53, and OR = 2.02, 95% CI = 1.32, 3.08, respectively), beginning of exposure in 1956+ and end of exposure in 1976+ (OR = 1.56, 95% CI = 1.21, 2.03, and OR = 1.5, 95% CI = 1.14, 1.98, respectively), and 1,000-49,999 driving hours (OR = 1.54, 95% CI = 1.15, 2.07). None of the ORs were significant in East Germany in this group.

For other traffic-related jobs (Group B) the overall OR was 1.53 (95% CI = 1.04, 2.24). The ORs for beginning of exposure in 1956+ and end of exposure in 1976+ were OR = 1.71, 95% CI = 1.05, 2.78, and OR = 2.68, 95% CI = 1.47, 4.90, respectively. The risk increased with

personal communication with Dr. Johnni Hansen confirmed that dieselization in Denmark was completed in the late 1940s and lorries, buses, and taxis have been using diesel fuel since then. Although direct adjustments were not done for smoking and exposure to asbestos, indirect information on both these confounders indicates that they are unlikely to explain the observed excesses and the increasing risk with increasing duration of employment. Thus, the results of this study are strongly supportive of DE being associated with increased lung cancer.

7.2.2.13. *Brüske-Hohlfeld et al. (1999): Lung Cancer Risk in Male Workers Occupationally Exposed to Diesel Motor Emissions in Germany*

This paper presents a pooled analysis of two case-control studies of lung cancer. The first study, by Jöckel et al. (1995, 1998), was conducted between 1988 and 1993 and had 1,004 cases and 1,004 controls matched for sex, age, and region of residence, selected randomly from the compulsory municipal registries. The inclusion criteria for cases were: they should have been born in or after 1913, should have been of German nationality, and should have been diagnosed with lung cancer within 3 months prior to the interview. The second study, by Wichmann et al. (1998), was ongoing when it was included in this study. The study span covered the years 1990 to 1996. By 1994 a total of 3,180 cases and 3,249 controls, randomly selected from the compulsory population registries, were frequency matched on sex, age, and region. The cases were less than 76 years old, were residents of the region and living in Germany for more than 25 years, and had a diagnosis not more than 3 months old. Of 4,184 pooled cases and 4,253 pooled controls, the analysis was conducted on 3,498 male cases and 3,541 male controls. A personal interview was conducted with each study participant. Data were collected on basic demographic information, detailed smoking history, and lifelong occupational history about jobs held and industries worked in. The job titles and industries were classified into 33 and 21 categories, respectively, using the German Statistical Office codes.

Based on job codes with potential exposure to diesel motor emission (DME), four exposure groups were constituted. Group A comprised professional drivers of trucks, buses, taxis, etc. Group B comprised other traffic-related jobs such as switchmen, diesel locomotive drivers, and diesel forklift truck drivers. Group C comprised bulldozer operators, graders, and excavators. Group D comprised full-time farm tractor drivers. Validation of the jobs was done by written evaluation of the job task descriptions, which also avoided misclassification. The following information was acquired for the construction of job task descriptions: (1) What were your usual tasks at work and how often (in % of daily working hours) were they performed? (2) What did you produce, manufacture, or transport? (3) Which material was used? (4) What kind of machine did you operate? Some individuals had more than one job task

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer

Authors	Population studied	DE exposure assessment	Results	Limitations
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
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 A 5-digit code was used to classify the occupations according to Nordic Classification of Occupations	For underground miners: SS OR = 2.7 (≥1 year of employment) SS OR = 9.8 (≥20 years of employment) For professional drivers: SNS OR = 1.2 (≥20 years of employment) with dead controls All ORs adjusted for smoking	Uncertain DE exposure No validation of exposure done Underground miners data not adjusted for other confounders such as radon, etc.

increasing duration of exposure and was statistically significant for >10-20 years (OR = 2.49) and more than 20 years (OR = 2.88). No separate analyses for West Germany and East Germany were presented in this category.

For heavy equipment operators (Group C) the overall OR of 2.31 (95% CI = 1.44, 3.7) was highest among all the job categories. Significant ORs were observed for beginning exposure in 1946-1955 (OR = 2.83, 95% CI = 1.10, 7.23) and end exposure in 1966-1975 (OR = 3.74, 95% CI = 1.20, 11.64). The risk increased with increasing duration of exposure and was statistically significant for more than 20 years of exposure (OR = 4.3). Although no separate analyses for West Germany and East Germany were presented, investigators mentioned that for this job group hardly any difference was seen between West Germany and East Germany.

For drivers of the farming tractors (Group D) the overall OR of 1.29 was not significant. Risk increased with increasing duration of exposure and was significant for exposure of more than 30 years (OR = 6.81, 95% CI = 1.17, 39.51). No separate analyses for West Germany and East Germany were presented in this category.

The professional drivers and the other traffic-related job categories probably have mixed exposures to gasoline exhaust in general traffic. On the other hand, it should be noted that exposure to DME among heavy equipment and farm tractor drivers is much higher and not as mixed as in professional drivers. The heavy equipment drivers usually drive repeatedly through their own equipment's exhaust. Therefore, the observed highest risk for lung cancer in this job category establishes a direct link with the DME. The only other study that found significantly higher risk for heavy equipment operators (RR = 2.6) was conducted by Boffeta et al. (1988). Although the only significant excess was observed for farming tractor operators among individuals with more than 30 years of exposure, a steady increase in risk was observed for this job category with increasing exposure. The investigators stated that the working conditions and the DME of tractors remained fairly constant over the years. This increase may be due mainly to exposure to DME and, in addition, PM_{10}.

This is a well-designed, well-conducted, and well-analyzed study. Its main strengths are large sample size, resulting in good statistical power; inclusion of incident cases that were diagnosed not more than 3 months prior to the interview; use of only personal interviews, reducing recall bias; diagnosis ascertained by cytology or histology; and availability of lifelong detailed occupational and smoking history. Exposure estimation for each individual was based on job codes and industry codes, which were validated by written job descriptions to avoid misclassification. The main limitation of the study is lack of data on actual exposure to DME. The cumulative quantitative exposures were calculated based on time spent in each job with potential exposure to DME and the type of equipment used. Thus, this study provides strong evidence for a causal association between exposure to DE and occurrence of lung cancer.

Table 7-2 summarizes the above lung cancer case-control studies.

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
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 Adjusted for age, smoking, & study area	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
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 Adjusted for age, smoking, & asbestos	Exposure based on job titles not validated Possible misclassification of exposure and smoking, based on next-of-kin information Lack of sufficient latency
Steenland et al. (1998)	Exposure-response analyses of their 1990 case-control study	Industrial hygiene data of elemental carbon in trucking industry collected by Zaebst et al. (1991) used to estimate individual exposures Cumulative exposures calculated based on estimated lifetime exposures	For mechanics: OR = 1.69 (had the highest DE exposure) Lowest DE exposure and lowest OR = 0.93 observed for dockworkers Increasing risk of lung cancer with increasing exposure Adjusted for age & smoking	

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
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 were observed for DE 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 Industrial hygiene sampling done	SS OR = 1.41 (≤64 year age group) SS OR = 1.64 (≤64 year age group) for ≥20 years DE exposure group when compared to 0- to 4-year exposure group All ORs adjusted for lifetime smoking and asbestos exposure	Probable misclassification of DE exposure jobs Years of exposure used as surrogate for dose 13% of death certificates not ascertained Overestimation of smoking history
Benhamou et al. (1988)	1,260 histologically confirmed lung cancer cases 2,084 non-tobacco-related disease matched controls (sex, age at diagnosis, hospital admission, and interviewer) Occurring between 1976 and 1980 in France	Based on exposures determined by panel of experts The occupations were recorded blindly using International Standard Classification of Occupations as chemical or physical exposures	Significant excess risks were found in motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35) (smoking adjusted)	Exposure based on occupational histories not validated Exposures classified as chemical and physical exposures, not specific to DE

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
Hansen et al. (1998)	Population-based case-control study of professional drivers in Denmark Male lung cancer cases diagnosed between 1970-1989, controls matched by year of birth and sex	Information about past employment obtained by linkage with nationwide pension fund Employment as lorry/bus drivers (n=1,640) and taxi drivers (n=426) was used as surrogate for exposure to DE	For lorry/bus drivers: SS OR = 1.31 For taxi drivers: SS OR = 1.64, which increased to 2.2 in > 5-year employment with no lag time & 3.0 in > 5 year employment with 10- year lag time SS trend test for increasing risk with increasing employment for both lorry/bus drivers & taxi drivers ($p<0.001$) All ORs adjusted for socioeconomic status	Lack of information on the type of fuel (personal communication with the principal investigator confirmed that diesel fuel is used for the lorry/buses and taxis since early 1960s) Even though direct adjustment was not done for smoking/asbestos, indirect methods indicate that the results are not likely to be confounded by these factors

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
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 DE	OR slightly below unity SNS Adjusted for smoking	No verification of exposure Duration of employment used as surrogate for dose Number of individuals exposed to DE 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 DE 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 Positive trend for DE observed (trend much steeper for smokers than nonsmokers) Adjusted for smoking	Numbers of cases and controls are small Very few nonsmokers Lack of exposure information on asbestos No latency analysis
Swanson et al. (1993)	Population based case-control study in metropolitan Detroit 3,792 lung cancer cases and 1,966 colon cancer (cases) controls, diagnosed between 1984 and 1987 in white and black males (aged between 40-84)	Telephone interviews with the individual or surrogate about lifetime work history Occupation and industry data coded per 1980 U.S. Census Bureau classification codes Certain occupations and industries were selected as unexposed to carcinogens	SS excess ORs observed for - black farmers OR= 10.4 for 20+ years employment - white railroad industry workers OR= 2.4 for 10+ years employment Among white trend tests were SS for -drivers of heavy duty trucks - drivers of light duty trucks - farmers - railroad workers Among blacks trend test was SS for farmers only All the ORs were adjusted for age at diagnosis, pack-years of cigarette smoking and race	Lack of direct information on specific exposures No latency analysis

7.2.3. Summaries of Studies and Meta-Analyses of Lung Cancer

7.2.3.1. *Cohen and Higgins (1995): Health Effects of DE: Epidemiology*

The Health Effects Institute (HEI) reviewed all published epidemiologic studies on the health effects of exposure to DE 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 DE 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 DE 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 1; 29 studies (83%) reported at least one relative risk greater than one 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 DE and lung cancer. They find that the evidence suggests that long-term exposure to DE 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 DE 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 based on 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 DE. 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

Table 7-2. Epidemiologic studies of the health effects of exposure to DE: case-control studies of lung cancer (continued)

Authors	Population studied	DE exposure assessment	Results	Limitations
Brüske-Hohlfeld et al. (1999)	Pooled analysis of two case-control studies (3,498 cases & 3,541 controls) Controls frequency matched on sex, age, & region, randomly selected from the compulsory population registry Inclusion criteria: (1) born in or after 1913/less than 75 years old, (2) German nationality/resident of the region - lived in Germany for more than 25 years, & (3) lung cancer diagnosis should be 3 months prior to the study Information obtained by personal interview on:	Lifetime detailed occupational & smoking histories obtained from each individual in a personal interview Based on job codes (33 job titles & 21 industries) potential DE exposure classified in 4 categories: A- professional drivers of trucks, buses, & taxis; B- other traffic related i.e., switchman, locomotive, & forklift drivers; C- bulldozer operators, graders,& excavators; D- farm tractor drivers Cumulative DE exposures and pack-years (smoking) calculated for each individual	SS higher risk adjusted for smoking observed for all 4 categories: A- ORs ranged from 1.25 to 2.53 B- ORs ranged from 1.53 to 2.88 C- ORs ranged from 2.31 to 4.3 D- 6.81 (exposure < 30 years) Risk increased with increasing exposure	Lack of data on actual exposure to diesel exaust

Abbreviations: OR = odds ratio; RR = relative risk; SNS = statistically nonsignificant; SS = statistically significant.

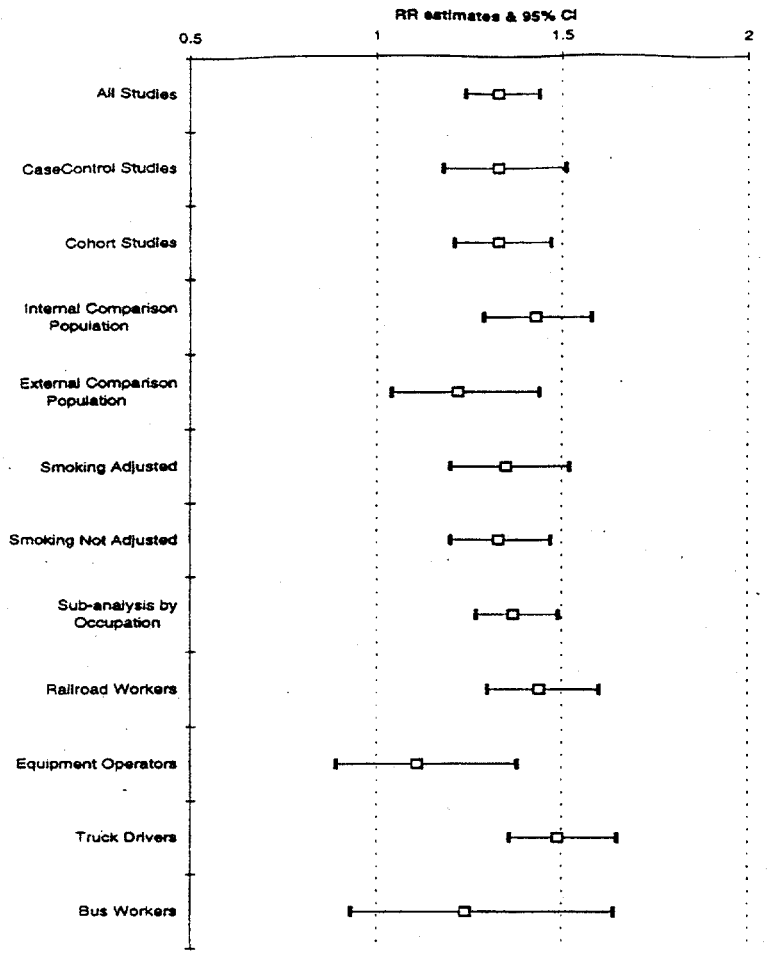


Figure 7-1. Pooled relative risk estimates and heterogeneity-adjusted 95% confidence intervals for all studies and subgroups of studies included in the meta-analysis.

Source: Bhatia et al., 1998.

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 the authors 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

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 studies may account for the consistent associations seen.

7.2.3.2. *Bhatia et al. (1998): DE Exposure and Lung Cancer*

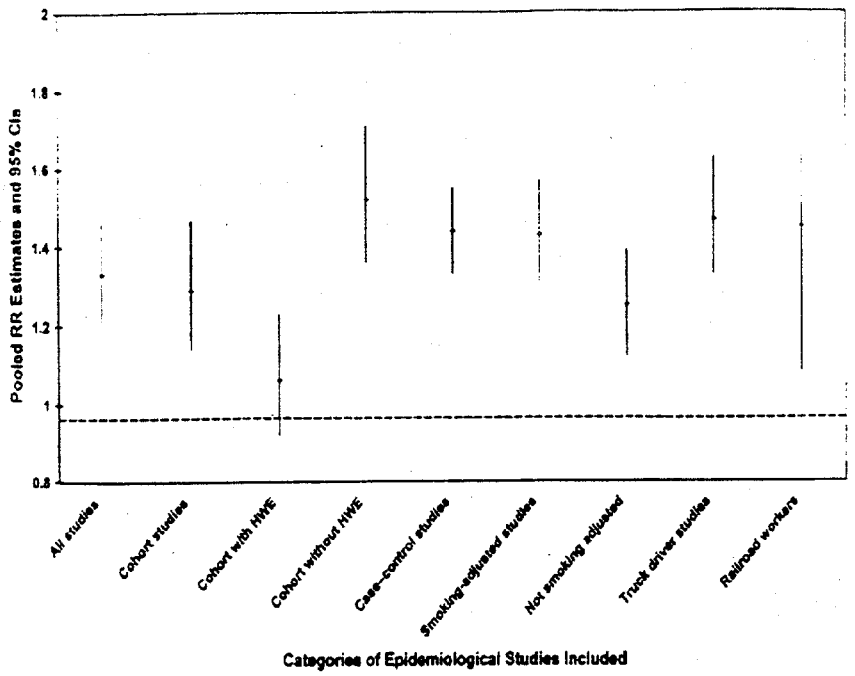
Bhatia et al. (1998) report a meta-analysis of 29 published[2] cohort and case-control studies of the relation between occupational exposure to DE and lung cancer. A search of the epidemiologic literature was conducted for all studies concerning lung cancer and DE exposure. Occupational studies involving 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 follow-up 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 DE-exposed workers, the subgroup risk estimates were used in the meta-analysis. Smoking-adjusted effect measures were used when present.

Of 29 studies 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), indicating increased relative risk for lung cancer from occupational exposure to DE. 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 DE. 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 DE exposure; and (3) in studies with internal

[2]Of 35 studies identified in the literature search, 6 pairs of studies represented analyses of the same study population, reducing the number of studies to 29.

populations most likely to have had substantial exposure to DE, the pooled smoking- adjusted relative risk was 1.47 (95% CI = 1.29, 1.67) (see Figure 7-2).



Note. CI = confidence interval; HWE = healthy worker effect.

Figure 7-2. Pooled estimates of relative risk of lung cancer in epidemiologic studies involving occupational exposure to DE (random-effects models).

Source: Lipsett and Campleman, 1999.

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 heterogeneity by subset analysis and linear meta-regressions. 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.

This meta-analysis also evaluated the potential for publication bias, which provides reassurance that the magnitude of published effects is not a function of the precision or study

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 DE exposure may be attributable to failure of adjustment rather than lack of confounding by cigarette smoking. A similar problem exists for the Bhatia et al. representation of the control for confounding in the study by Boffetta and Stellman (1988).

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.

7.2.3.3. *Lipsett and Campleman (1999): Occupational Exposure to DE 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 DE 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 follow-up 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

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 DE. All studies have relied on the job categories or self-report of exposure to DE. Gustavsson et al. (1990), Emmelin et al. (1993), and Brüske-Hohlfeld et al. (1999) estimated exposure levels by getting detailed histories of job tasks/categories and computing cumulative exposures, which unfortunately were not verifiable due to of the lack of industrial hygiene data. In the studies by Garshick et al. (1987, 1988), the diesel-exhaust-exposed job categories were verified based on 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 DE 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 DE exposure. Unfortunately, the exposure was only qualitatively verified. Quantitative use of this information would have been much more meaningful. Zaebst et al. (1991) conducted an industrial hygiene survey of elemental carbon exposure in the trucking industry by job categories. Using these exposure measurements, Steenland et al. (1998) conducted an exposure-response analysis of their earlier lung cancer case-control study (Steenland et al., 1990). These exposure data are currently being verified and will be used for quantitative risk assessment in the near future.

Occupations involving potential exposure to DE are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators. No known studies in metal miners have assessed whether DE is associated with lung cancer. Currently, there are about 265 underground metal/nonmetal mines in the United States (Department of Labor, Mine Safety and Health Administration, 2001). Approximately 20,000 miners are employed, but not all of them are currently working in the mines. Diesel engines were introduced in metal mines in the United States 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 in the United States, and their use is still quite limited. There are 910 underground coal mines in the United States, of which only 145 currently use diesel powered equipment (Department of Labor, Mine Safety and Health Administration, 2001). Even if it were possible to estimate how many miners (metal and coal) were exposed to DE, 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.

power. Again, as stated in the Bhatia et al. (1998) review, 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.

7.2.4. Summary and Discussion

Certain extracts of DE have been demonstrated as both mutagenic and carcinogenic in animals and in humans. Animal data suggest that DE is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods of time. While rat lung cancer response to DE is not suitable for dose-response extrapolation to humans, the positive lung cancer response doses imply a hazard for humans. Because large working populations are currently exposed to DE and because nonoccupational ambient exposures currently are of concern as well, the possibility that exposure to this complex mixture may be carcinogenic to humans has become an important public health issue.

Because diesel emissions become diluted in the ambient air, it is difficult to study the health effects in the general population. Nonoccupational exposure to DE 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 DE. 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 likely to be underestimations of true risk.

A major difficulty with the occupational studies considered here was measurement of actual DE exposure. Because all the cohort mortality studies were retrospective, assessment of health effects from exposure to DE 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 DE with either standard populations (presumably no exposure to DE) or men in other job categories from industries with little or no potential for DE exposure. A few studies have included measurements of diesel fumes, but there is no

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 or refute a causal association between exposure to DE 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 DE. 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). Recently Brüske-Hohlfeld et al. (1999) also have observed significantly higher risk for lung cancer, in the range of 2.31 to 4.3, for heavy equipment operators. 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 DE 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 DE 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 DE and asbestos exposures. Statistically significant increasing lung cancer relative risks of 1.34, 1.81, and 2.43 were observed for DE 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 low power (small cohort) and lack of smoking histories. But smoking is not likely to be different among study individuals irrespective of their exposure status to DE.

Hansen (1993), on the other hand, found statistically significant SMR of 160 from 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 DE was assumed in truck drivers for diesel-powered trucks, but no validation of exposure was attempted. Follow-up period was short, no latency analysis was done, and smoking data were lacking. However, a population survey carried out in 1988 showed very little difference in smoking habits of residents of rural area and the total Danish male population, thus, smoking is unlikely to confound the finding of

Both metal and coal mines in Europe and Australia, on the other hand, have been using diesel equipment for more than 50 years. The epidemiologic studies of coal miners conducted in these countries discuss only exposures to coal dust. In most of the coal miner studies, DE exposures are not even mentioned by the investigators as confounding exposures. Therefore, it is not known how many miners, if any, were exposed to DE, for how long, and at what concentrations. Although studies of coal miners reviewed by IARC (1997) generally found lower than expected lung cancer mortality (with some exceptions where some excess of lung cancer was observed), without knowing the concentrations, duration of exposure, and number of miners exposed to DE, it is inappropriate to conclude that the reported lung cancer mortality deficit in these studies provides a proof positive of absence of causal association between DE exposure and occurrence of lung cancer.

7.2.4.1. *Summary of the Cohort Mortality Studies*

The cohort studies mainly demonstrated an increase in lung cancer. Studies of bus company workers by Waller (1981), Rushton et al. (1983), and Edling et al. (1987) failed to demonstrate any statistically significant excess risk of lung cancer, but these studies have certain methodological problems, such as small sample sizes, short follow-up periods (just 6 years in the Rushton et al. study), lack of information on confounding variables, and lack of analysis by duration of exposure, duration of employment, or latency that preclude their use in determining the carcinogenicity of DE. Although the Waller (1981) study had a 25-year follow-up period, the cohort was restricted to employees (ages 45 to 64) currently in service. Employees who left the job earlier, as well as those who were still employed after age 64 and who may have died from cancer, were excluded.

Wong et al. (1985) conducted a mortality study of heavy equipment operators that demonstrated a 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 DE exposure. One of the methodologic limitations of this study is that most of these men worked outdoors; thus, this cohort might have had relatively low exposure to DE. 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.

lung cancer. On the other hand, an analysis of the same data by California EPA (CalEPA, 1998) yielded a positive dose response set using age at 1959 and adding an interaction term of age and calendar year in the model. However, Crump (1999) reported that the negative dose-response continued to be upheld in his latest analysis when age was controlled more carefully and years of exposure quantified more accurately. Crump (1999) asserted that the negative dose-response trends for lung cancer observed with either the cumulative exposure or duration of exposure may be due to underascertainment of deaths in the last 4 years of follow-up of the Garshick et al. (1988) study, as well as incomplete follow-up in earlier years. The HEI (1999) special panel conducted its own analyses using Garshick et al. (1988) data to evaluate their usefulness for quantitative risk assessment and found results similar to those of Crump et al. (1991) and Garshick (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991). The HEI panel reported consistently elevated risk of lung cancer for train workers compared with clerks for each duration of employment, and that shop workers had an intermediate risk of lung cancer. But they found decreasing risk of lung cancer with increasing duration of employment. The panel discussed various possibilities (different types of biases) for the negative dose-response and advised against using the Garshick et al. (1988) data for quantitative risk assessment. The panel also reported the strengths of the Garshick et al. (1988) study such as large population, control for asbestos, and smoking, and concluded that the study was generally consistent with findings of weak association between exposure to DE and occurrence of lung cancer. Hence, the divergent results of these recent analyses do not negate the positive evidence this study provides for the qualitative evaluation. The observance of dose-response would have strengthened the causal association, but an absence of a dose-response does not negate it.

Suggestive evidence is provided by a recent study of potash miners in Germany. The information on the exposure (including elemental carbon and organics), work chronology, and work category was used by the investigators to calculate cumulative exposures for each worker. Furthermore, information on smoking habits indicated homogeneity in the cohort.
A statistically nonsignificant twofold increase in lung cancer was observed in the production workers as compared to workshop workers. The lack of significance for this finding could be due to short follow-up, not enough latency, and relatively young age of the cohort.

7.2.4.2. *Summary of the Case-Control Studies of Lung Cancer*

Among the 11 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

excess lung cancer. The findings of both these studies are consistent with the findings of other truck driver studies and are supportive of causal association.

Two mortality studies of railroad workers were conducted by Howe et al. (1983) and Garshick et al. (1988). The Howe et al. study, which was conducted in Canada, 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 DE and the risk of lung cancer. The main limitation of the study was the inability to separate overlapping exposures of coal dust/combustion fumes and DE fumes. Information on jobs was available at retirement only. There also was insufficient detail on the classification of jobs by DE 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/combustion fumes and DE fumes and not just one or the other. It should be noted that, so far, coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners. However, lack of data on confounders such as asbestos and smoking (though use of the internal comparison group to compute relative risks minimizes confounding by smoking) makes interpretation of this study difficult. When three DE 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 DE being a lung carcinogen.

The strong evidence for linking DE 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. The investigators reported that the risk of lung cancer increased with increasing duration of employment. As this was a large cohort study with a lengthy follow-up and adequate analysis, including dose response (based on duration of employment as a surrogate) as well as adjustment for other confounding factors such as asbestos, the observed association between increased lung cancer and exposure to DE is more meaningful. Even though the reanalysis of these data by Crump et al. (1991) found that the relative risk could be positively or negatively related to duration of exposure depending on how age was controlled, additional analysis by Garshick et al. (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991) found that the relationship between years exposed when adjusted for the attained age and calendar years was flat to negative, depending on the choice of the model. They also found that deaths were underreported by approximately 20% to 70% between 1977 and 1980, and their analysis based on job titles, limited to 1959-1976, showed that the youngest workers still had the highest risk of dying of

observed in this study. Of 50 cases and 154 controls, only 6 individuals were nonsmokers. Although intricate exposure matrices were created using three different variables, no direct exposure measurement was done. Despite the limitations of small number of cases and controls; lack of data on asbestos exposure, which is fairly common in dockworkers; and very few nonsmokers; this study provides consistent support for a real effect of DE exposure and occurrence of lung cancer, at least in smokers.

The most convincing evidence comes from the case-control studies among railroad workers by Garshick et al. (1987); among truck drivers of the Teamsters Union by Steenland et al. (1990, 1998); among truck drivers, railroad workers, and farmers in a population-based study by Swanson et al. (1993); among different professional drivers in Denmark by Hansen et al. (1998); and among male workers occupationally exposed to diesel motor emissions in Germany by Brüske-Hohlfeld et al. (1999). Garshick et al. (1987) 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 DE exposure, the risk of lung cancer increased with duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent DE exposure (5 years before death), the relative odds increased to 1.43 (95% CI = 1.06, 1.94). This appears to be a well-conducted and well-analyzed study with reasonably good power. Potential confounders were controlled adequately, and interactions between DE and other lung cancer risk factors were tested. Some of the limitations of this study are misclassification of exposure because ICC job classification was used as surrogate for exposure and use of death certificates for identification of cases and controls.

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 main strengths of the study are availability of detailed records from the Teamsters Union, a relatively large sample size, availability of smoking data, and measurements of exposure. The limitations of this study include possible misclassifications of exposure and smoking, lack of levels of diesel exposure, a smaller nonexposed group, and an insufficient latency period. Recently Steenland et al. (1998) conducted an exposure-response analysis on these cases and controls, using the industrial hygiene survey results of Zaebst et al. (1991). The estimates were made for long-haul drivers, short-haul drivers, dockworkers, mechanics, and those outside the trucking industry. The survey found that mechanics had the

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 DE. On the other hand, statistically nonsignificant excess risks were observed for DE exposure by Hall and Wynder (1984) in workers who were exposed to DE 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. Hall and Wynder (1984) had a high nonparticipation rate of 36%. Therefore, the positive results found in this study 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 exposures to DE from those to 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 DE. 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.

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). A strong interaction between smoking and DE was

Brüske-Hohlfeld et al. (1999) recently conducted a pooled analysis of two case-control studies among male workers occupationally exposed to DME in Germany. The investigators collected data on demographic information, detailed smoking, and occupational history. Job titles and industries were classified in 33 and 21 categories respectively. Job descriptions were written and verified to avoid misclassification of estimated exposure to diesel emissions. Individual cumulative DME exposures and smoking pack-years were calculated. Asbestos exposures were estimated by certain job-specific supplementary questions. Analysis of 3,498 lung cancer cases and 3,541 controls yielded statistically significant ORs ranging from 1.25 to 2.31 adjusted for smoking and asbestos exposure. The risk increased with increasing years of exposure for both the first year of exposure and the end year of exposure. These investigators presented analyses by various job categories, by years of exposure, first and end years of exposure and, when possible, separately for West and East Germany. Significantly higher risks were found among all four job categories. For professional drivers (of trucks, buses, and taxis) ORs ranged from 1.25 to 2.53. For other traffic-related jobs (switchmen, diesel locomotive drivers, diesel forklift truck drivers), ORs ranged from 1.53 to 2.88. For heavy equipment operators (bulldozers, graders, and excavators), ORs ranged from 2.31 to 4.3, and for drivers of farming equipment the only significant excess (OR = 6.81) was for exposure for <30 years.

This study shows increased risk for all the DME-exposed job categories. The professional drivers and the other traffic-related jobs also have some mixed exposures to gasoline exhaust in general traffic. On the other hand, it should be noted that exposure to DME among heavy equipment and farm tractor drivers is much higher and not as mixed as in professional drivers. The heavy equipment drivers usually drive repeatedly through their own equipment's exhaust. Therefore, the observed highest risk for lung cancer in this job category establishes a strong link with the DME. The only other study that found significantly higher risk for heavy equipment operators (RR = 2.6) was conducted by Boffeta et al. (1988). Although the only significant excess in the group was observed for farming tractor operators with more than 30 years of exposure, a steady increase in risk was observed for this job category with increasing exposure. The investigators stated that the working conditions and the DME of tractors remained fairly constant over the years. This increase may be due mainly to exposure to DME and PM_{10}.

The main strengths of the study are large sample size, resulting in good statistical power; inclusion of incident cases diagnosed not more than 3 months prior to the interview; use of only personal interviews, reducing recall bias; diagnoses ascertained by cytology or histology; and availability of lifelong detailed occupational and smoking history. Exposure estimation done for each individual was based on job codes and industry codes, which were validated by written job descriptions to avoid misclassification.

highest current levels of DE exposures and dockworkers who mainly used propane- powered forklifts had the lowest exposure. The finding of the highest lung cancer risk for mechanics and lowest for dock workers is indicative of a causal association between the DE exposure and development of lung cancer. However, the risk among mechanics did not increase with increasing duration of employment. The ORs for quartile cumulative exposures, computed by using logistic regression adjusted for age, race, smoking, diet, and asbestos exposure, showed a pattern of increasing trends in risk with increasing exposure, between 1.08 and 1.72 depending upon exposure level and lag structure used.

In a population-based lung cancer case-control study Swanson et al. (1993) found statistically significant excess risks adjusted for age at diagnosis, smoking, and race, among white male drivers of heavy trucks employed for ≥20 years and railroad workers employed for ≥10 years (OR = 2.5, 95% CI = 1.1, 4.4, and OR = 2.4, 95 % CI = 1.1, 5.1, respectively), and among black farmers employed for ≥20 years (OR = 10.4, 95% CI = 1.4, 77.1). Although individual ORs were not significant for various occupations with potential exposure to DE, statistically significant trends were observed for drivers of heavy trucks, light trucks, farmers, and railroad industry workers among whites, and among black farmers ($p \leq 0.05$). The main strengths of the study are availability of data on lifetime work history and smoking history; the main limitation is absence of actual specific exposure data. This is the first study that found increased lung cancer risk for farmers, who are exposed to DE of their farm tractors.

Hansen et al. (1998), in their study of professional drivers in Denmark, found statistically significant ORs (adjusted for socioeconomic status) of 1.31, 1.64, and 1.39 for lorry/bus drivers, taxi drivers, and unspecified drivers, respectively. The lag time analyses for duration of employment were unchanged for lorry/bus drivers but increased to OR = 3 from 2.2 in taxi drivers with a lag time of 10 years and duration of employment of > 5 years. The authors asserted that the higher risk seen in the taxi drivers may be due to higher exposure to these drivers because of longer time spent in traffic congestion. Furthermore, the trend tests for increasing risk of lung cancer with increasing duration of employment were statistically significant for both lorry/bus drivers and taxi drivers in both 10-year lag time and no lag time. The main strengths of the study are the large sample size, availability of detailed employment records, and information on socioeconomic status. The main limitations are absence of individual data on smoking habits and asbestos exposure, and information about the type of fuel used for the vehicles driven by these professional drivers. A personal communication with the main investigator revealed that the lorries/buses and taxis have been using diesel fuel since the late 1940s. Moreover, indirect information about smoking and asbestos exposure indicated that these two confounders are unlikely to explain the observed excesses or the trends, resulting in strong support of earlier positive studies.

(1997) call into question the assertions by Cohen and Higgins (1995), Bhatia et al. (1998), and Lipsett and Campleman (1999) that the associations seen for DE 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, and thus, the observed association between exposure to DE and excess risk of lung cancer is more likely to be due to bias. 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.

7.2.4.4. *Discussion of Relevant Methodologic Issues*

A persistent association of risk for lung cancer and DE exposure has been observed in more than 30 epidemiologic studies published in the literature over the past 40 years. Evaluation of whether this association can be attributed to a causal relation between DE exposure and lung cancer requires careful consideration of whether chance, bias, or confounding might be likely alternative explanations.

A total of 10 cohort and 12 case-control studies are reviewed in this chapter. An increased lung cancer risk was observed in 8 cohort and 10 case-control studies, even though the results were not always statistically significant. There is a consistent tendency for point estimates of relative risk to be greater than one in studies that adjusted (either directly or indirectly) for smoking, had a long enough follow-up, and sufficient statistical power among truck drivers, railroad workers, dock workers, and heavy equipment workers. If this elevated risk was due to chance one would expect almost equal distribution of these point estimates to be above and below one. 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 DE 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 DE 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 DE and lung cancer. Several occupational epidemiologic

The main limitation of the study is lack of data on actual exposure to DME. The cumulative quantitative exposures were calculated based on time spent in each job with potential exposure to DME and the type of equipment used. Thus, this study provides strong evidence for causal association between exposure to DE and occurrence of lung cancer.

7.2.4.3. *Summary of the Reviews and Meta-Analyses of Lung Cancer*

Three summaries of studies concerned with the relationship of DE exposure and lung cancer risk are reviewed. The HEI report is a narrative study of 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 excess 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 DE 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 or 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 DE. 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 DE 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 DE 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 DE exposure. On the other hand, Stöber and Abel (1996), Muscat and Wynder (1995), and Cox

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 DE because most of the increase in lung cancer would be attributed to smoking rather than to exposure to DE.

Some studies do not adjust for tobacco smoke exposure. Even though smoking is a strong risk for lung cancer, it is only a confounder if there are differential smoking habits among individuals exposed to DE versus individuals who are not exposed. Most of the occupational cohorts include workers from the same socioeconomic background or used an internal comparison group; hence, it is unlikely that confounding by cigarette smoking is substantial in these studies. Some studies have adjusted for socioeconomic status and some studies have compared the cigarette smoking habits by conducting rural and urban general population surveys. Besides, in studies with long enough latency, adjustment for cigarette smoking did not alter substantially the observed higher risk.

Another methodologic concern in these studies is use of death certificates to determine cause of death. Death certificates were used by all of the cohort mortality studies and some of the case-control studies of lung cancer to determine cause of death. Use of death certificates could lead to misclassification bias because of overdiagnosis. 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. These more recent findings suggest that the diagnosis of lung cancer on death certificates is better than anticipated. In reality, lung cancer is one cause of death that has been found to be generally reliably reported on the death certificate. Thus, the misclassification bias probably is minimal in the studies described in this chapter.

Finally, several investigators have not conducted latency analysis in their studies. The latent period for lung cancer development is from 20 to 30 years or more. Considering the fact that dieselization was not complete till almost 1959 for locomotives and the 1970s for the trucking industry in the United States, most of the cohort studies conducted in the U.S. population do not have a long enough follow-up period to allow for latency of 20 to 30+ years. In addition, the study inclusion criteria for most of the studies are individuals who worked in the industry for at least 6 months /1 year from the beginning of the follow-up period to the end of

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 consistent association seen in the aggregate data. Given the variety of designs used in studying the DE 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 consistent association seen. Exposure information bias is certainly a problem for almost all of the studies concerned. Detailed and reliable individual-level data on DE 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. The variability in actual lifetime exposure to DE in an occupational cohort may not be reflected in differences in job title, and there might be considerable variability in actual exposure despite similar job titles. 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 DE–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 consistent aggregate association observed.

Moreover, throughout this chapter, various methodologic limitations of individual studies have been discussed, such as small sample size, short follow-up period, lack of data on confounding variables, use of death certificates to identify the lung cancer cases, and lack of latency analysis. The studies with small sample sizes (i.e., not enough power) and short follow-up periods (i.e., not enough latent period) have been difficult to interpret due to these limitations.

The most important confounding variable is smoking which is a strong risk factor for lung cancer. All the studies considered for this report are either cohort retrospective mortality or case-control studies where history of exposures in the past is elicited. 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

et al., 1983; Wong et al., 1985; Gustavsson et al., 1990; Emmlin et al., 1993; Hansen, 1993; Hansen et al., 1998) and, after adjustment for smoking and/or asbestos, RRs and ORs remained statistically significant and in the same range in certain studies (Dambar and Larson 1987; Garshick et al., 1987, 1988; Benhamou et al., 1988; Boffetta and Stellman, 1988; Hays et al., 1989; Steenland et al., 1990; Swanson et al., 1993; Brüsk-Hohlfeld et al., 1999). In addition, two meta-analyses demonstrated that not only did excess in lung cancer remain the same after stratification/adjustment for smoking and occupation, but in several instances the pooled RRs showed modest increases, with little evidence of heterogeneity. Overall, the studies in epidemiologic terms show relatively modest to weak association between DE and occurrence of lung cancer. Even though strong associations are more likely to be causal than modest-to-weak associations, the fact that association is relatively modest or weak does not rule out the causal link.

- *Consistency.* Increased lung cancer risk has been observed in several cohort and case-control studies, conducted in several industries and occupations in which workers were potentially exposed to DE. However, not all the excesses were statistically significant. Statistically significant lung cancer excesses adjusted for smoking were observed in truck drivers (Hayes et al., 1989; Hansen, 1993; Swanson et al., 1993; Brüske-Hohlfeld et al., 1999), professional drivers (Benhamou et al., 1988; Brüske-Hohlfeld et al., 1999), railroad workers (Garshick et al., 1987; Swanson et al., 1993), heavy equipment drivers (Boffetta and Stellman, 1988; Brüske-Hohlfeld et al., 1999), and farm tractor drivers (Swanson et al., 1993; Brüske-Hohlfeld et al., 1999). Furthermore, the two recent meta-analyses by Bhatia et al. (1998) and Lipsett and Campleman (1999) found that even though a substantial heterogeneity existed in their initial pooled estimates, stratification on several factors demonstrated a relationship between exposure to DE and excess lung cancer that remained positive throughout various analyses.

- *Specificity.* This criterion requires that a single cause lead to a single effect. With respect to exposure to DE, excess for lung cancer is the only effect that is found to be consistently elevated and statistically significant in several studies. Quite a few studies have examined DE for other effects such as bladder cancer, leukemia,

the follow-up period. Hence, the later the individual enters the cohort, the shorter the follow-up period; thus, the latent period is insufficient for the occurrence of lung cancer in these late entrants. Therefore, the observed slight to moderate increase in risk of lung cancer could be due to insufficient latency. On the other hand, in certain case-control studies the elapsed period between the identification of the lung cancer cases and exposure to DE is long enough to allow for the 30+ years latency needed for the development of lung cancer (Hansen et al., 1998; Brüske-Hohlfeld et al., 1999). These investigators identified lung cancer cases in the early to mid-1990s and found significant excess risks for lung cancer among the individuals exposed to DE. It should be noted that the use of diesel fuel for trucks, buses, and taxis had started in their countries (Denmark and Germany, respectively) in the late 1940s.

7.2.4.5. *Evaluation of Causal Association*

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 used the original or modified version of a set of criteria provided by Hill (1965)[3] 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 Hill criteria (1965) of causal inference, as modified by Rothman (1986), to the studies reviewed here resulted in the following:

- *Strength of association.* This phrase refers to the magnitude of the ratio of incidence or mortality (RRs or ORs). Several studies found statistically significant RRs and ORs that ranged from 1.2 to 2.6 (Howe et al., 1983; Rushton

[3]Hill in his address to the Royal Society of Medicine in 1965 on "The environment and disease: association or causation" explored several aspects of association between exposure and occurrence of an event before deciding that the most likely interpretation of it is causation. He provided nine different aspects of association that he characterized as his viewpoints before interpreting the association being causal. The epidemiologic community universally adopted these (aspects/viewpoints) later as criteria for causality/causal association.

First, DE has been shown to cause lung and other cancers in animals (Heinrich et al., 1986b; Iwai et al., 1986b; Mauderly et al., 1987; Pott et al., 1990; Mauderly, 1994). Second, it contains highly mutagenic substances such as polycyclic aromatic hydrocarbons as well as nitroaromatic compounds (Claxton, 1983; Ball et al., 1990; Gallagher et al., 1993; Sera et al., 1994; Nielsen et al., 1996a) that are recognized human pulmonary carcinogens (IARC, 1989). Third, DE consists of carbon core particles with surface layers of organics and gases; the tumorigenic activity may reside in one, some, or all of these components. As explained in Chapter 4, there is clear evidence that the mixture of organic constituents, both in particles and vapor phases, 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. Further, increased levels of peripheral blood cell DNA adducts associated with occupational exposure to DE have been observed in humans (Nielsen et al., 1996a,b). Thus, the above evidence makes a convincing case that occupational exposures to DE are causally associated with the occurrence of lung cancer is highly plausible biologically.

In conclusion, the epidemiologic studies of exposure to DE and occurrence of lung cancer furnish evidence that is consistent with a causal association. This association observed in several studies is unlikely to be due to chance or bias. Although many studies did not have information on smoking, significant confounding by smoking is unlikely in these studies because the comparison population was from the same socioeconomic class. The strength of association (i.e., RRs/ORs between 1.2 and 2.6) was weak to modest by epidemiologic standards, with dose-response relationships observed in several studies. Last, but not least, there is highly plausible biological evidence that exposure to DE could result in excess risk of lung cancer in humans.

7.3. CARCINOGENICITY OF DIESEL EXHAUST IN LABORATORY ANIMALS

This chapter summarizes studies that assess the carcinogenic potential of DE 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) DE and unfiltered (whole) DE to differentiate gaseous-phase effects from effects induced by diesel PM (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 DE and tumor initiators, tumor promoters, or cocarcinogens.

Particulate matter concentrations in the DE used in these studies ranged from 0.1 to 12 mg DPM /m^3. In this chapter, any mention of statistical significance implies that $p \leq 0.05$ was reported in the reviewed publications. A summary of the animal inhalation carcinogenicity studies and their results is presented in Table 7-3.

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 7-4 and 7-5. 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, polycyclic aromatic hydrocarbons [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 DE 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 months. The animals were exposed in 3,000 L plexiglass chambers. Airflow was equal to 50 liters per minute. Chamber temperatures were maintained between 25 °C and 26.5 °C. Relative humidity ranged from 45% to 80%. Exposures were carried out during the daytime. The 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 months exposure for gross necropsy and histopathological examination.

The only tumor detected was a bronchiolar adenoma in the group exposed over 16 months to DE. No lung tumors were reported in controls. The equivocal response may have been caused by the relatively short exposure durations (20 months) 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 months.

Table 7-3. 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 M, 40	Clean air Whole exhaust	8.3	None None	6 hr/day, 5 days/ week, for up to 20 mo	NA	0/6 (0) 1/6 (16.6)	
								Bronchoalveolar carcinoma	
Kaplan et al. (1983) White et al. (1983)	Rat/F344	M, 30 M, 30 M, 30 M, 30	Clean air Whole exhaust Whole exhaust Whole exhaust	0 0.25 0.75 1.5	None None None None	20 hr/day, 7 days/ week, for up to 15 mo	8 mo 8 mo 8 mo 8 mo	0/30 (0) 1/30 (3.3) 3/30 (10.0) 1/30 (3.3)	
								Adenomas	Carcinomas	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b) Mohr et al. (1986)	Rat/ Wistar	F, 96 F, 92 F, 95	Clean air Filtered exhaust Whole exhaust	4	None None None	19 hr/day, 5 days/ week for up to 35 mo	NA	0/96 (0) 0/92 (0) 8/95 (8.4)	0/96 (0) 0/92 (0) 0/95 (0)	0/96 (0) 0/92 (0) 9/95 (9.4)	0/96 (0) 0/92 (0) 17/95 (17.8)[c]	
								Adenomas	Adenocarcinoma and adenosquamous carcinoma	Large cell and squamous cell carcinomas	All tumors	
Iwai et al. (1986a,b)	Rat/F344	F, 24 F, 24 F, 24	Clean air Filtered exhaust Whole exhaust	4.9	None None None	8 hr/day, 7 days/ week, for 24 mo	NA	1/22 (4.5) 0/16 (0) 3/19 (0)	0/22 (0) 0/16 (0) 3/19 (15.8)	0/22 (0) 0/16 (0) 2/19 (10.5)	1/22 (4.5)[d] 0/16 (0) 8/19 (42.1)[c,e]	

Table 7-3. 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
								Adenoma	Carcinoma			
Takemoto et al. (1986)	Rat/F344	F, 12 F, 21 F, 15 F, 18	Clean air Clean air Whole exhaust Whole exhaust	0 0 2-4 2-4	None DIPN[h] None DIPN[h]	4 hr/day, 4 days/ week, 18-24 mo	NA	0/12 (0) 10/21 (47.6) 0/15 (0) 12/18 (66.7)	0/12 (0) 4/21 (19) 0/15 (0) 7/18 (38.9)			
								Adenomas	Adenocarcinoma + squamous cell carcinoma	Squamous cysts	All tumors	
Mauderly et al. (1987)	Rat/F344	M + F, 230[b] M + F, 223 M + F, 221 M + F, 227	Clean air Whole exhaust Whole exhaust Whole exhaust	0 0.35 3.5 7.1	None None None None	7 hr/day, 5 days/ week up to 30 mo	NA	(0) (0) (2.3) (0.4)	(0.9) (1.3) (0.5) (7.5)	(0) (0) (0.9) (4.9)	(0.9) (1.3) (3.6)[c] (12.8)[c]	
								Adenomas	Adenosquamous carcinomas	Squamous cell carcinomas	All tumors	
Ishinishi et al. (1988a) Heavy-duty engine	Rat/F344	M + F, 123 M + F, 123 M + F, 125 M + F, 123 M + F, 124	Clean air Whole exhaust Whole exhaust Whole exhaust Whole exhaust	0 0.5 1.0 1.8 3.7	None None None None None	16 hr/day, 6 days/ week, for up to 30 mo	NA	0/123 (0) 0/123 (0) 0/125 (0) 0/123 (0) 0/124 (0)	1/123 (0.8) 0/123 (0) 0/125 (0) 4/123 (3.3) 6/124 (4.8)	0/123 (0) 1/123 (0.8) 0/125 (0) 0/123 (0) 2/124 (1.6)	1/123 (0.8) 1/123 (0.8) 0/125 (0) 4/123 (3.3) 8/124 (6.5)[c]	

Table 7-3. 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	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		0.1	None		12 mo	0/8 (0)	0/8 (0)	0/8 (0)	
		NS, 11		0.1	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)	
		NS, 5		1.1	None		6 mo	0/5 (0)	0/5 (0)	0/5 (0)	
Light duty		NS, 9	Whole exhaust	1.1	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)	
		NS, 11		1.1	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)	
			Whole exhaust								
			Whole exhaust								
			Whole exhaust								
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		0.5	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)	
		NS 11		0.5	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)	
		NS, 5		1.8	None		6 mo	0/5 (0)	0/5 (0)	0/11 (0)	
		NS, 6		1.8	None		12 mo	0/6 (0)	0/6 (0)	0/6 (0)	
		NS, 13		1.8	None		18 mo	0/13 (0)	1/13 (0)	1/13 (0)	
			Whole exhaust								
			Whole exhaust								
			Whole exhaust								

Table 7-3. 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
								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)		Tumor incidence for all rats dying or sacrificed
		M + F, 144	Filtered exhaust (medium exposure)	0	None			0/144 (0)		
		M + F, 143	Filtered exhaust (high exposure)	0	None			0/143 (0)		
		M + F, 143	Whole exhaust	0.7	None			1/143 (0.7)		♀ 24/25 (96%) after 24 mo ♂ 12/27 (44%) after 24 mo
		M + F, 144	Whole exhaust	2.2	None			14/144 (9.7)[c]		
		M + F, 143	Whole exhaust	6.6	None			55/143 (38.5)[c]		
								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]				(93.8)	
		F, NS	Whole exhaust	4.2	DPN[e]			(16.7)	(89.6)	
		F, NS	Filtered exhaust	0	DPN[e]			(31.3)[c]	(89.6)	
								(14.6)		
Lewis et al. (1989)	Rat/F344	M + F, 288[a]	Clean air	2	None	7 hr/day, 5 days/ week, 24 mo	NA	No tumors	0/192 (0)	
			Whole exhaust		None				0/192 (0)	

Table 7-3. 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
								Adeno-squamous 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		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)	
			Whole exhaust									
			Whole exhaust									
								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		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		11.6	None			13/100 (13)	13/100 (13)	4/100 (4)	20/100 (20)	incidences
		F, 100	Whole exhaust	10.0	None			4/100 (4)	13/100 (13)	3/100 (3)	20/100 (20)	after 30 mo
			Carbon black									
			TiO_2									
								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		6.5	None			23/212 (11)	22/212 (10)	3/212 (1)	1/212 (<1)	0/212 (0)
		M + F, 213	Whole exhaust	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)
			Carbon black									

Table 7-3. 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
Iwai et al. (1997)	F/344	121, F 108, F 153, F	Clean air Filtered air Whole exhaust	0 0 3.2-9.4	None None None	NA 48-56 hr/day 48-56 hr/day	NA 6 mo 6 mo	5/121(4%) type not stated 2/108(4%) type not stated 53/153(35%) 61.3% adenoma, 25.8% adenocarcinoma, 2.2% benign squamous cell tumor, 7.5% squamous cell carcinoma, 3.2% adenosquamous carcinoma	Cumulative exposure dose ranged from 154-274 mg/m^3
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

Table 7-3. 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
								Lung tumors	
	Mouse/ Jackson A	M + F, 40	Clean air	0	None	20 hr/day, 7 days/ week,	8 weeks	16/36 (44.4)	0.5 tumors/ mouse
		M + F, 40	Whole exhaust	6.4	None	for 8 weeks	8 weeks	11/34 (32.3)	0.4 tumors/ mouse
	Mouse/ Jackson A	F, 60	Clean air	0	None			4/58 (6.9)	0.09 tumors/ mouse
		F, 60	Clean air	0	Urethan[l]	20 hr/day, 7 days/ week, for approx.		9/52 (17.3)	0.25 tumors/ mouse
		F, 60	Whole exhaust	6.4	None	7 mo.		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 M, 18 M, 485	Clean air Clean air Whole exhaust	1.5	None Urethan[k] None	20 hr/day, 7 days/ week, for 3 mo	6 mo	Pulmonary adenomas 144/458 (31.4) 18/18 (100) 165/485 (34.2)	
Kaplan et al. (1983) White et al. (1983)	Mouse/ A/J	M, 388 M, 388 M, 399 M, 396	Clean air Whole exhaust Whole exhaust Whole exhaust	0 0.25 0.75 1.5	None None None None	20 hr/day, 7 days/ week, for up to 8 mo	NA	Pulmonary adenoma 130/388 (33.5) 131/388 (33.8) 109/399 (27.3) 99/396 (25.0)	

Table 7-3. 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	Carcinomas	All tumors	
Pepelko and Peirano (1983)	Mouse/ Sencar	M + F, 260	Clean air	121212	None	Continuous for 15 mo	NA	(5.1)	(0.5)	(5.6)	
			Clean air		BHT[j]			(12.2)	(1.7)	(2.8)	
			Clean air		Urethan[k]			(8.1)	(0.9)	(9.0)	
			Whole exhaust		None			(10.2)[c]	(1.0)	(11.2)[c]	
			Whole exhaust		BHT[j]			(5.4)	(2.7)	(8.1)	
			Whole exhaust		Urethan[k]			(8.7)	(2.6)	(11.2)	

Table 7-3. 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
								All tumors	
Pepelko and Peirano (1983)	Mouse/ Strain A	M + F, 90	Clean air	1212012	None		NA	21/87 (24)	0.29 tumors/ mouse
			Clean air		Exposure (darkness)			59/237 (24.9)	0.27 tumors/ mouse
			Whole exhaust		Exposure (darkness)			10/80 (12.5)	0.14
			Whole exhaust		Urethan[m]			22/250 (0.10)	0.10
			Clean air		Urethan[m]			66/75 (88)	2.80
			Whole exhaust					42/75 (0.95)	0.95

								Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors	
Heinrich et al. (1986a,b)	Mouse/ NMRI	M + F, 84	Clean air	4	None	19 hr/day, 5 days/ week for up to 30 mo	NA	9/84 (11)	2/84 (2)	---	11/84 (13)	
		M + F, 93	Filtered exhaust		None			11/93 (12)	18/93 (19)[c]	---	29/93 (31)[c]	
		M + F, 76	Whole exhaust		None			11/76 (15)	13/76 (17)[c]	---	24/76 (32)[c]	
Takemoto et al. (1986)	Mouse/ IRC	M + F, 45	Clean air	0	None	4 hr/day, 4 days/ week, for 19-28 mo	NA					
		M + F, 69	Whole exhaust	2-4	None							
									Adenoma	Adenocarcinoma		
	Mouse/ C57BL	M + F, 12	Clean air	0	None	4 hr/day, 4 days/ week for 19-28 mo	NA		3/45 (6.7)	1/45 (2.2)		
		M + F, 38	Whole exhaust	2-4	None				6/69 (8.7)	3/69 (4.3)		

Table 7-3. 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/6N	F, 120	Clean air	4.5	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
		F, 120	Whole exhaust		None			8/38 (21.1)	3/38 (7.9)				8.5% tumor rate
		F, 120	Particle-free exhaust		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)				
				11.6	None			(21.8)	(15.4)				
			Carbon black	10	None			(11.3)	(10)				
			TiO_2					(11.3)	(2.5)				
	Mouse/ NMRI	F, 120	Clean air	4.5	None	18 hr/day, 5 days/ week, 23 mo	None	(25)	(8.8)				
		F, 120	Whole exhaust		None			(18.3)	(5.0)				
		F, 120	Particle-free exhaust		None			(31.7)	(15)				
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	Multiple adenomas	Multiple carcinomas	Adenomas/ carcinoma	Alveolar/ bronchiolar adenoma	Alveolar/ bronchiolar carcinoma	
		M + F, 171	Whole exhaust	0.35	None			1/157 (0.6)	2/157 (1.3)	1/157 (0.6)	10/157 (6.4)	7/157 (4.5)	
		M + F, 155	Whole exhaust	3.5	None			2/171 (1.2)	1/171 (0.6)	1/171 (0.6)	16/171 (9.4)	5/171 (2.9)	
		M + F, 186	Whole exhaust	7.1	None			0/155 (0)	1/155 (0.6)	0/155 (0)	8/155 (5.2)	6/155 (3.9)	
								0/186 (0)	0/186 (0)	0/186 (0)	10/186 (5.4)	4/186 (2.2)	
Heinrich et al. (1986a,b)	Hamster/ Syrian	M + F, 96	Clean air		None	19 hr/day 5 days/week for up to 30 mo		Adenomas	Adenocarcinoma	Squamous cell tumors	All tumors		
		M + F, 96	Filtered exhaust		None			0/96(0)	0/96(0)	0/96	0/96(0)		
								0/96(0)	0/96(0)	0/96	0/96(0)		
		M + F, 96	Whole exhaust	4	None		NA	0/96(0)	0/96(0)	0/96	0/96(0)		

Table 7-3. 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
								Primary lung tumors	
Brightwell et al. (1989)	Hamster/ Syrian Golden	M + F, M + F, 202	Clean air Clean air	0 0	None DEN[j]	16 hr/day, 5 days/ week, for 24 mo	NA	7/202 (3.5) 4/104 (3.8)	Respiratory tract tumors not related to exhaust exposure for any of the groups
		M + F, 104	Filtered exhaust (medium dose)	0	DEN[j]			9/104 (8.7)	
		M + F, 104	Filtered exhaust (high dose)	0	DEN[j]			2/101 (2.0)	
		M + F, 101	Whole	0.7	DEN[j]			6/102 (5.9)	
		M + F, 102	exhaust	2.2	DEN[j]			4/101 (3.9)	
		M + F, 101	Whole	6.6	DEN[j]			1/204 (0.5)	
		M + F, 204	exhaust	0	None			0/203 (0)	
		M + F, 203	Whole exhaust Filtered exhaust (high dose) 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]Dipentylnittrasamine; 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/m^3 from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 mg/m^3.
[n]120-121 males and 71-72 females examined histologically.
[o]Not all animals were exposed for full term, at least 10 males were killed at 3, 6, and 12 mo of exposure.
NS = Not specified.
NA = Not applicable.

Table 7-4. Tumor incidences in rats following intratracheal instillation of DE 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

Table 7-5. Tumorigenic effects of dermal application of acetone extracts of 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.

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 months. 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 months. 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.

Although five instances of bronchoalveolar carcinoma were observed in 90 rats exposed to DE for 15 months and held an additional 8 months 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 months did not exhibit any carcinomas. No tumors were observed in any of the 180 rats exposed to DE for 9 or 15 months 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 months and held an additional 8 months 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 DE 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 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 of Toxicology and Aerosol Research, female Wistar rats were exposed for 19 hr/day, 5 days/week to both filtered and unfiltered (total) DE 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 DE 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 unfiltered DE. Squamous metaplasia was observed in 65.3% of the rats breathing unfiltered DE, 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. (1986b) examined the long-term effects of DE 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 DE, filtered DE, or filtered room air for 8 hr/day, 7 days/week for 24 months. 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-months control rat, providing a spontaneous tumor incidence of 4.5%. No lung tumors were observed in rats exposed to filtered DE. 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 months 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 DE. 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 DE was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-3. 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 DE. In addition, splenic malignant lymphomas occurred during exposure to both filtered and unfiltered DE. This is the only report to date of tumor induction at an extrarespiratory site by inhaled DE in animals.

A chronic (up to 24 months) inhalation exposure study was conducted by Takemoto et al. (1986), in which female Fischer 344 rats were exposed to DE 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. 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 DE 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 of 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 months 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 tailpipe, 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. No exposure-related changes in body weight or lifespan 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 7-3. 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 months 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 DE 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-months whole-body exposure protocol (16 h/day, 6 days/week) used DPM concentrations of 0, 0.5, 1, 1.8, or 3.7 mg/m^3 from HD engines and 0, 0.1, 0.4, 1.1, or 2.3 mg/m^3 from LD engines. The 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 7-3.

The Ishinishi et al. (1988a) study also included recovery tests in which rats exposed to whole DE (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 months were examined for lung tumors following 6-, 12-, or 18-month 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 DE and not provided a recovery period. The only carcinoma observed was in a rat examined 12 months following exposure to exhaust (1.8 mg/m^3) from the HD engine.

Brightwell et al. (1986, 1989) studied the effects of DE on male and female F344 rats. The DE 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) DE 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 7-3). DE-induced tumor incidence in rats was dose-related and higher in females than in males (Table 7-3). 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 DE 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 months 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 DE 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 DE and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole DE (2 mg soot/m^3), coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), or DE 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 DE 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.

Histological examination was performed on 120 to 121 male and 71 to 72 female rats terminated after 24 months 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 DE (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 maintained at 23-25 °C and 50%-70%, respectively. DE 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 7-3). 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 DE. 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 DE 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%. DE was generated with 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 DE 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 DE), and 12.3 (high DE). The percentages of rats (males and females combined) with malignant or benign neoplasms were 1.4 (control), 6.2 (low DE), and 17.9 (high DE). 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 7-3. 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 months 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 DE 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 months and examined for lung tumors. In the present series, inhalation exposure was substituted. DE 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 DE (to simulate chemical reactions induced by sunlight) or nonirradiated DE (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 DE, then held in clean air until sacrificed at 9 months of age. No tumorigenic effects were detected at 9 months 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 months of age and approximately 100 at 12 months 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 DE,

however, significantly inhibited the tumorigenic effects of the 5-mg urethan treatment. Results are listed in Table 7-3.

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 months of age. Briefly, the animals were exposed in inhalation chambers to DE 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. Temperature 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 DE. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to DE for 8 months (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 7-3).

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. 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 months 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 DE. Male and female parent-generation mice were exposed to DE 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 DE 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 months old.

The incidence of pulmonary adenomas (16.3%) was significantly increased in the mice exposed to DE 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 DE. 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 because they are also sensitive to induction of skin tumors. These data are summarized in Table 7-3.

Takemoto et al. (1986) reported the effects of inhaled DE (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. All numbers reported are for males and females combined. Four adenomas and 1 adenocarcinoma were detected in 34 DE-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 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 (1990a) indicated that the difference in combined benign and malignant tumors between whole DE-exposed C57BL/6N mice and corresponding controls was significant at $p<.05$. See Table 7-3 for details of tumor incidence.

Heinrich et al. (1986b) and Stöber (1986), as part of a larger study, also evaluated the effects of DE 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 DE 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 months to whole DE diluted to produce a particle concentration of 4.5 mg/m^3. These data are summarized in Table 7-3.

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 DE (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. DE 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 °C-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 months 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.

Although 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 DE exposure on tumor frequency in female Syrian golden hamsters. Groups of 48 to 72 animals were exposed to clean air or whole DE 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 DE 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 DE-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 DE 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 DE on male and female Syrian golden hamsters. Groups of 52 males and 52 females, 6 to 8 weeks old, were exposed to DE 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. No statistically significant (t test) relationship between tumor incidence and exhaust exposure was reported.

In summary, DE 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 DE (2 mg/m^3) for 7 hr/day, 5 days/week for 24 months (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), DE 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 DE exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo time frame 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 DE)

Several studies have been conducted in which animals were exposed to DE filtered to remove PM. As 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. 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., 1986a), 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. (1986a) 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 DE in laboratory animals at concentrations tested.

7.3.3. Inhalation Studies (DE plus Cocarcinogens)

Details of the studies reported here have been described earlier and in Table 7-3. 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 DE up to 12 mg/m^3 (Pepelko and Peirano, 1983).

Heinrich et al. (1986b) exposed Syrian hamsters of both sexes to DE 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 B[*a*]P. Female NMRI mice received weekly intratracheal instillations of 50 or 100 μg B[*a*]P 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 DE. Response to B[*a*]P did not differ from that of BaP alone in hamsters, but results were inconsistent in mice. Although 20 B[*a*]P instillations induced a 71% tumor incidence in mice, concomitant diesel exposure resulted in only a 41% incidence. However, neither 10 B[*a*]P instillations nor DBA instillations induced significant effects.

Takemoto et al. (1986) exposed Fischer 344 rats for 2 years to DE 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 DE.

Brightwell et al. (1989) investigated the concomitant effects of DE and DEN in Syrian hamsters exposed to DE 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 DE alone. Heinrich et al. (1989b) also exposed Syrian hamsters of both sexes to DE 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 DE.

Heinrich et al. (1989a) investigated the effects of DPN in female Wistar rats exposed to DE 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 DE. 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 DE. 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 DE-exposed animals.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990b), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole DE, 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 DE; 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 DE 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 7-6). 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 DE on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with DPM (1 mg/week for 10

Table 7-6. Tumor incidence and survival time of rats treated by surgical lung implantation with fractions from DE 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)	6.7	97 (24-139)	0	1	0
Hydrophobic fraction (II) (75)	20.00	99 (50-139)	50601	1000	14.2
Nonaromatics +					
PAC[c] 2 + 3 rings (IIa) (72)	19.22	103 (25-140)			0
PAH[d] 4 to 7 rings (IIb) (0.8)	0.21	102 (50-140)			17.1
Polar PAC (IIc) (1.1)	0.29	97 (44-138)			0
Nitro-PAH (IId) (0.7)	0.19	106 (32-135)			2.8
Reconstituted hydrophobics (Ia, b, c, d) (74.5)	19.91	93 (46-136)	70027113	101000	20.0
Control, unrelated		110 (23-138)			0
Control (beeswax/trioctanoin)		103 (51-136)			0
B[*a*]P	0.3	69 (41-135)			77.1
	0.1	98 (22-134)			31.4
	0.03	97 (32-135)			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.

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 months were autopsied. All animals surviving 30 months or more postinstillation were sacrificed and evaluated for histopathology. Among 42 animals exposed to DPM surviving 18 months 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 months and those sacrificed at 30 months were not reported separately. Statistical analysis indicated that activated carbon induced a significant increase in lung tumor incidence compared with no tumors in 50 uninjected controls and 1 tumor in 23 solvent-injected controls. The tumor incidence 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 DE 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), 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, whereas B[*a*]P was effective only at high doses. Total dose and tumor responses are shown in Table 7-4.

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 DE 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 DE.

The effects on hamsters of intratracheally instilled DPM suspension, DPM with Fe_2O_3, or DPM extract with Fe_2O_3 as the carrier were studied by Shefner et al. (1982). The DPM component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0 mg/week for 15 weeks to groups of 50 male Syrian golden hamsters. The total volume instilled was 3.0 mL (0.2 mL/week for 15 weeks). The DPM and dichloromethane extracts were suspended in physiological saline with gelatin (0.5% w/v), gum arabic (0.5% w/v), and propylene glycol (10% by volume). The Fe_2O_3 concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles (Fe_2O_3) without the DPM component. Two replicates of the experiments were performed. Adenomatous hyperplasia was reported to be most severe in those animals treated with DPM or DPM plus Fe_2O_3 particles and least severe in those animals receiving DPM plus Fe_2O_3. Of the two lung adenomas detected microscopically, one was in an animal treated with a high dose of DPM and the other was in an animal receiving a high dose of DPM extract. Although lung damage was increased by instillation of DPM, there was no evidence of tumorigenicity.

7.3.4.3. *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 months 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 months 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 7-5. In the initial 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 months 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 months 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 7-7. The tumor initiation-promotion assay was considered positive if a dose-dependent response was obtained and if at least two doses provided a papilloma-per-mouse value that was three times or greater than that of the background value. Based on these criteria, only emissions from the Nissan were considered positive. Tumor initiation and complete carcinogenesis assays required that at least one dose produce a tumor incidence of at least 20%. None of the DPM samples yielded positive results based on this criterion.

Kunitake et al. (1986, 1988) evaluated the effects of a dichloromethane extract of DPM obtained from a 1983 MMC M-6D22P 11-L V-6 engine. An acetone solution was applied in 10 doses every other day, followed by promotion with 2.5 μg of TPA three times weekly for 25 weeks. Exposure groups received a total dose of 0.5, 5, 15, or 45 mg of extract. Papillomas were reported in 2 of 50 animals examined in the 45 mg exposure group and in 1 of 48 in the 15 mg group compared with 0 of 50 among controls. Differences, however, were not statistically significant.

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 DE, the relevant mode for human exposure, had been reported. Since then long-term inhalation bioassays with DE 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.

Table 7-7. 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.

It can be reasonably concluded that with adequate exposure, inhalation of DE 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 7-8 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. However, studies such as those reported by Driscoll et al. (1996) support the existence of a threshold if it is assumed that inflammation is a prerequisite for lung tumor induction. 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.

Although 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, 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 DE 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, 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.

Table 7-8. Cumulative (concentration × time) exposure data for rats exposed to whole DE

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. (1987)	35, 30	4.20042004e+15	0	0	14701470029820	0.9
	35, 30		0.35	12.25		1.3
	35, 30		3.5	122.5		3.6
	35, 30		7.1	248.5		12.8
Nikula et al. (1995)	80, 23	736073607360	0	0	1840047840	1.0
	80, 23		2.5	200.0		7.0
	80, 23		6.5	520.0		18.0
Heinrich et al. (1986a)	95, 35	1330013300	4.24	402.8	56392	17.8
	95, 35					
Heinrich et al. (1995)	90, 24	8.64086409e+15	0	0	74002180061700	0
	90, 24		0.8	72.0		0
	90, 24		2.5	225.0		5.5
	90, 24		7.0	630.0		22.0
Ishinishi et al. (1988a)	96, 30	1.15201152e+49	0	0	1.1524	3.3
(Light-duty engine)	96, 30		0.1	9.6	60813e+37	2.4
	96, 30		0.4	38.4		0.8
	96, 30		1.1	105.6		4.1
(Heavy-duty engine)	96, 30		2.3	220.8		2.4
	96, 30		0	0		0.8
	96, 30		0.5	48.0		0.8
	96, 30		1.0	96.0		0
	96, 30		1.8	172.8		3.3
	96, 30		3.7	355.2		6.5

Table 7-8. Cumulative (concentration × time) exposure data for rats exposed to whole DE (continued)

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	
Brightwell et al. (1989)	80, 24	7.6807681e+15	0	0	53761689650688	1.2
	80, 24		0.7	56.0		0.7
	80, 24		2.2	176.0		9.7
	80, 24		6.6	528.0		38.5
Kaplan et al. (1983)	140, 15	8.4008401e+15	0	0	2100630012600	0
	140, 15		0.25	35.0		3.3
	140, 15		0.75	105.0		10.0
	140, 15		1.5	210.0		3.3
Iwai et al. (1986b)	56, 24	53765376	4.9	274.4	26342	36.8
	56, 24					
Takemoto et al. (1986)	16, 18-24	1,152-1,536	0	0	0	0
	16, 18-24	1,152-1,536	2-4	32-64	3,456-4,608	
Karagianes et al. (1981)	30, 20	24002400	8.3	249	19920	16.6
	30, 20					
Iwai et al. (1997)	56, 24	537649925616	9.4	526154275	5.47041597e+14	421242
	48, 24		3.2			
	54, 24		5.1			

Attempts to induce significant increases in lung tumors in Syrian hamsters by inhalation of whole DE 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., 1989) developed tumors following 2-year exposure to DE. 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 DE filtered to remove particulate matter failed to induce lung tumors in rats (Heinrich et al., 1986b; Iwai et al., 1986b; 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), 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 DE 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 DE. 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., 1985; Bond et al., 1990) affirm observations of the potential carcinogenicity of DE by providing evidence for DNA damage in rats. These findings are discussed in more detail in Chapter 3, 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 (1990b) 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 studies in rats exposed to high concentrations, intratracheal instillation studies in rats and mice exposed to high doses, and supported by positive mutagenicity studies, the evidence for carcinogenicity of DE is considered to be adequate in animals. The contribution of the various fractions of DE 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 long-term exposure at high concentrations of poorly soluble 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 DE 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 EXHAUST-INDUCED CARCINOGENESIS

As noted in Chapter 2, 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. Because exposure to the vapor phase alone, even at high concentrations, failed to induce lung cancer in laboratory animals (Heinrich et al., 1986b), the mode-of-action discussion will focus on the particulate matter phase. Additive or synergistic effects of vapor-phase components, however, cannot be ruled out, as chronic inhalation bioassays involving exposure to diesel particles alone have not been carried out.

Several hypotheses regarding the primary mode of action of DE 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, 1990b). 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 DE 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. Because 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 DE are B[*a*]P, dibenz[*a,h*]anthracene, pyrene, chrysene, and nitroarenes such as 1-NP, 1,3-DNP, 1,6-DNP, and 1,8-DNP, all of which are mutagenic, carcinogenic, or implicated as procarcinogens or cocarcinogens (Stenback et al., 1976; Weinstein and Troll, 1977; Thyssen et al., 1981; Pott and Stöber, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988). 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 DE.

Grimmer et al. (1987) separated DE 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 DE. 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 B[*a*]P 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%). Because 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). Although this study shows the tumor-inducing capability of PAHs resulting from combustion, it should be noted that the B[*a*]P 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 DE, 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 worth noting 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, as 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 polymorphonucleocytes (PMNs) (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to B[*a*]P; at 0.05 to 0.5 micromolar concentration, 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 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 that might be effective at amplifying any potential DE 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 DE 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 DE 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 DE when compared with 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 DE exposure compared with unexposed mechanics. Hou et al. (1995) found elevated adduct levels in bus maintenance workers exposed to DE. Although no difference in mutant frequency was observed between the groups, the adduct levels were significantly different (3.2 vs. 2.3 $\times$ 10^{-8}). Nielsen et al. (1996b) 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 in Rats by Diesel Exhaust

It is well recognized that the deposition of particles in the lung can result in the efflux of PMNs from the vascular compartment into the alveolar space compartment in addition to expanding the AM population size. Following acute exposures, the influx of the PMNs is transient, lasting only a few days (Adamson and Bowden, 1978; Bowden and Adamson, 1978; Lehnert et al., 1988). During chronic exposure the numbers of PMNs lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled 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., 1997a). 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 rodent 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).

Driscoll et al. (1997b) have demonstrated that exposure to doses of particles producing significant neutrophilic inflammation are associated with increased mutation in rat alveolar type II cells. The ability of particle-elicited macrophages and neutrophils to exert a mutagenic effect on epithelial cells in vitro supports a role for these inflammatory cells for the in vivo mutagenic effects of particle exposure. The inhibition of bronchoalveolar lavage cell-induced mutations by catalase implies a role for cell-derived oxidants in this response.

Hatch and co-workers (1980) have demonstrated that interactions of guinea pig AMs with a wide variety of particles, such as silica, metal oxide-coated fly ash, polymethylmethacrylate beads, chrysotile asbestos, fugitive dusts, polybead carboxylate microspheres, glass and latex beads, uncoated fly ash, and fiberglass increase the production of reactive oxygen species. Similar findings have been reported by numerous investigators for human, rabbit, mouse, and guinea pig AMs (Drath and Karnovsky, 1975; Allen and Loose, 1976; Beall et al., 1977; Lowrie and Aber, 1977; Miles et al., 1977; Rister and Baehner, 1977; Hoidal et al., 1978). 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 (e.g., superoxide [O_2^-] and hydroxyl radical [$^{\cdot}OH$] 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 with that generated via an enzymatic route (Ichinose et al., 1997a). They reported that O_2^- and $^{\cdot}OH$ 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 $^{\cdot}OH$. 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 although 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, whereas 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. Although adducts were not measured in this study, it nevertheless provides support for the likelihood that 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 correlates 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 DE.

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). 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). Comparison of several chronic inhalation studies correlating particle mass and particle surface area retained in the lung with tumor incidence indicated that particle surface area is a much better dosimeter than particle mass (Oberdörster and Yu, 1990; Driscoll et al., 1996). Heinrich et al. (1995) also found that lung tumor rates increased with specific particle surface area following exposure to DE, 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. Toxicity and carcinogenicity increased with decreasing particle size into the submicron range. For example, Heinrich et al. (1995) have shown that ultrafine titanium dioxide (approximately 0.2 μm diameter) is much more toxic than particles with a 10-fold greater diameter of the same composition used in an earlier study by Lee et al. (1986). This increase in toxicity has been noted with even smaller particles. For example, carbon black particles 20 nm in diameter were shown to be significantly more toxic than 50 nm particles (Murphy et al., 1999). The relationship between particle size and toxicity is of concern because, as noted in Chapter 2, approximately 50%-90% of the number of particles in DE are in the size range from 5 to 50 nm. 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

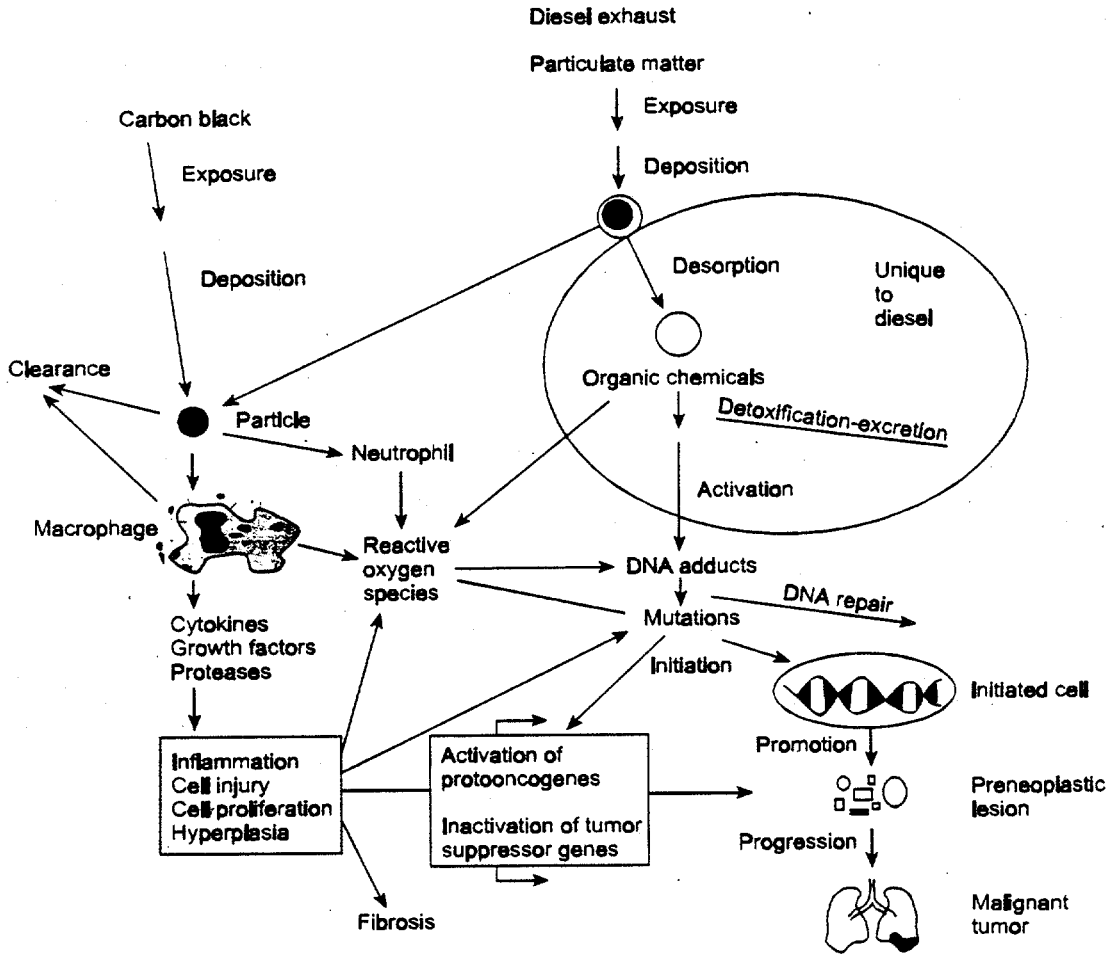
The induction of lung cancer in rats by large doses of carbon black via inhalation (Heinrich et al., 1995; Mauderly et al., 1991; Nikula et al., 1995) or intratracheal instillation (Kawabata et al., 1994; Pott et al., 1994; Dasenbrock et al., 1996) led to the development of the lung particle overload hypothesis. According to this hypothesis the induction of neoplasia by insoluble low- toxicity 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 7-3). 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 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 exposure concentrations, the lung particle overload condition is not present and the overload-induced inflammatory effects are not present. Note, however, as discussed in Chapters 5 and 6, that other types of inflammation are present in the rat lung at exposures below those inducing lung particle overload. However, at low exposures, activation of organic carcinogens and generation of oxidants from the organic fraction can still be expected. Actual contribution depends upon elution/bioavailability and the effectiveness of antioxidants. Direct effects of ultrafine diesel particles taken up by epithelial cells are also likely to play a role.

Although 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, humans and rats both develop an alveolar macrophage response, accumulate particles in the interstitium, and show mild interstitial fibrosis (ILSI, 2000). 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 with 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 the possible greater sensitivity of pulmonary response seen after longer exposures at high doses make pulmonary histopathology in rats a valid basis for noncancer dose-response assessment.

Figure 7-3. Pathegenesis of lung disease in rats with chronic, high-level exposures to particles.



Source: Modified from McClellan, 1997.

7.4.6. Summary

Recent studies have shown rat lung tumor rates resulting from exposures to nearly organic-free carbon black (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 also biological plausibility for lung cancer induction in humans at concentrations insufficient to induce lung particle overload as seen in rats (Chapter 3, Section 3.4 and ILSI, 2000). The 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 may play a role in this response and provide a basis for the plausibility. The slower particle clearance rates in humans (up to a year or more) may result in greater extraction of organics. This is supported by reports of increased DNA adducts in humans occupationally exposed to DE at concentrations unlikely to induce lung particle overload. Although 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 DE and other particles at high exposure concentrations may be species-specific and not DPM specific. The likelihood that different modes of action predominate at high and low doses, such as lung particle overload, also renders high-dose extrapolation to lower ambient concentrations uncertain.

7.5. WEIGHT-OF-EVIDENCE EVALUATION FOR POTENTIAL HUMAN CARCINOGENICITY

A carcinogenicity weight-of-evidence evaluation is a synthesis of all pertinent information addressing the question of how likely an agent is to be a human carcinogen. EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) provide a classification system for the characterization of the overall weight of evidence for potential human carcinogenicity based on human evidence, animal evidence, and other supportive data. This system includes Group A: *Human Carcinogen*; Group B: *Probable Human Carcinogen*; Group C: *Possible Human Carcinogen*; Group D: *Not Classifiable as to Human Carcinogenicity*; and Group E: *Evidence for Noncarcinogenicity to Humans.*

As part of the guidelines development and updating process, the Agency has developed revisions to the 1986 guidelines to take into account knowledge gained in recent years about the carcinogenic processes. With regard to the weight-of-evidence evaluation for potential human carcinogenicity, EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996b) and the subsequent revised external review draft (U.S. EPA, 1999) emphasize the need for characterizing cancer hazard, in addition to hazard identification. To express the weight of evidence for potential human carcinogenicity, EPA's proposed 1996 and 1999 guidelines utilize a hazard narrative in place of the 1986 A-E classification system. In order to provide some measure of consistency in using the 1996 and 1999 draft guidelines, standard hazard descriptors are used as part of the hazard narrative. The revised guidelines also stress the importance of considering the mode(s) of action information for making an inference about potential cancer hazard beyond the range of observation, typically encountered at levels of exposure in the general environment. "Mode of action" refers to a series of key biological events and processes that are critical to the development of cancer. This is contrasted with "mechanisms of action," which is defined as a more detailed description of the complete sequence of biological events at the molecular level that must occur to produce a carcinogenic response.

The sections to follow evaluate and weigh the individual lines of evidence and combine all evidence to make an informed judgment about the carcinogenicity hazard of DE. A conclusion in accordance with EPA's 1986 classification system (U.S. EPA, 1986) is provided, as well as a hazard narrative along with appropriate hazard descriptors according to EPA's Proposed Guidelines (U.S. EPA, 1996b, 1999). These sections draw on information reviewed in Chapters 2, 3, 4, and 7.

7.5.1. Human Evidence

Twenty-two epidemiologic studies about the carcinogenicity of workers exposed to DE in various occupations are reviewed in Section 7.2. Exposure to DE has typically been inferred based on job classification within an industry. Increased lung cancer risk, although not always statistically significant, has been observed in 8 out of 10 cohort and 10 of 12 case-control studies within several industries, including railroad workers, truck drivers, heavy equipment operators, and professional drivers. The increased lung cancer relative risks generally range from 1.2 to 1.5, though a few studies show relative risks as high as 2.6. Statistically significant increases in pooled relative risk estimates (1.33 to 1.47) from two independent meta-analyses further support a positive relationship between DE exposure and lung cancer in a variety of DE-exposed occupations.

The generally small increased lung cancer relative risk (less than 2) observed in the epidemiologic studies and meta-analyses potentially weakens the evidence of causality. When a relative risk is less than 2, if confounders (e.g., smoking, asbestos exposure) are having an effect on the observed risk increases, it could be enough to account for the increased risk. With the

strongest risk factor for lung cancer being smoking, there is a concern that smoking effects may be influencing the magnitude of the observed increased relative risks. However, in studies for which the effects of smoking were accounted for, increased relative risks for lung cancer prevailed. Though some studies did not have information on smoking, significant confounding by smoking is unlikely because the comparison populations were from the same socioeconomic class. Moreover, when the meta-analysis focused only on the smoking-controlled studies, the relative risks tended to increase.

As evaluated in Section 7.2.4.5, application of the criteria for causality (including the biological plausibility) leads to the conclusion that the increased risks observed in available epidemiologic studies are consistent with a causal association between exposure to DE and occurrence of lung cancer. Overall, the human evidence for potential carcinogenicity for DE is judged to be strong, but less than sufficient for DE to be considered as a human carcinogen because of exposure uncertainties (lack of historical exposure data for workers exposed to DE) and an inability to reach a fully and direct accounting for all possible confounders.

7.5.2. Animal Evidence

DE and its organic constituents, both in the gaseous and particle phase, have been extensively tested for carcinogenicity in many experimental studies using several animal species and with different modes of administration.

Several well-conducted lifetime rat inhalation studies have consistently demonstrated that chronic inhalation exposure to sufficiently high concentrations of DE produced dose-related increases in lung tumors (benign and malignant). However, the lung cancer responses in rats from high-concentration exposures appear to be mediated by impairment of lung clearance mechanisms through particle overload, resulting in persistent chronic inflammation and subsequent pathologic and neoplastic changes in the lung. Overload conditions are not expected to occur in humans as a result of environmental or most occupational exposures to DE. Thus, the rat lung tumor response is not considered relevant to an evaluation of the potential for a human environmental exposure-related hazard (Section 7.4).

The chronic inhalation studies of DE in mice showed equivocal results, whereas negative findings were consistently seen in hamsters. The gaseous phase of DE (filtered exhaust without particulate fraction) was found not to be carcinogenic in rats, mice, or hamsters.

In several intratracheal instillation studies, diesel particulate matter (DPM), carbon black, and the organic DPM extracts which were virtually devoid of PAHs, have been found to produce increased lung tumors in rats. When directly implanted into the rat lung, DPM condensate containing mainly four- to seven-ring PAHs induced increases in lung tumors. In several dermal studies in mice, DPM extracts have also been shown to cause skin tumors and sarcomas in mice following subcutaneous injection.

Available data and hypotheses suggest that both the carbon core and the adsorbed organics have potential roles in inducing lung tumors in the rat, although their relative contribution to the carcinogenic response remains to be determined.

The consistent findings of carcinogenic activity by DPM and the organic extracts of DPM in noninhalation studies (intratracheal instillation, lung implantation, skin painting) contribute to the overall evidence for a human hazard potential for DE. The lack of a tumor response from traditional animal inhalation studies in other rodent species is noted. Without understanding the mode(s) of action of DE's carcinogenicity in humans it is difficult to assess the meaning of nonpositive results from the mouse and hamster inhalation bioassays, and the unusable results from the rat, while having other evidence of carcinogenic potential and plausibility.

It should be noted that the animal studies used DE from engines available in the 1980s, and that present-day engine emissions have different characteristics (e.g., higher elemental carbon content and lesser amounts of adsorbed organics on the carbon particles), with uncertain impact on the outcome of the experimental studies. The same point can be made for the occupational epidemiologic studies.

7.5.3. Other Key Data

Other key data are judged to be supportive of potential carcinogenicity of DE. As discussed in Chapter 2, DE is a complex mixture of hundreds of constituents in either gaseous phase or particle phase. Although present in small amounts, several organic compounds in the gaseous phase (e.g. PAHs, formaldehyde, acetaldehyde, benzene, 1,3-butadiene) are known to exhibit mutagenic and/or carcinogenic activities. PAHs and PAH derivatives, including nitro-PAHs, present on the diesel particle are also known to be mutagenic and carcinogenic. As reviewed in Chapter 4, DPM and DPM organic extracts have been shown to induce gene mutations in a variety of bacteria and mammalian cell test systems. In addition, DE, DPM and DPM extracts have been found to cause chromosomal aberrations, aneuploidy, and sister chromatid exchange in both in vivo and in vitro tests.

There is also suggestive evidence for the bioavailability of the organics from DE (Chapter 3, Section3.5). Elevated levels of DNA adducts in lymphocytes have been reported in workers exposed to DE. In addition, animal studies showed that some of the radiolabeled organic compounds are eluted from DE particles following deposition in the lungs.

7.5.4. Mode of Action

As discussed in Section 7.4, the modes of action of DE-induced carcinogenicity in humans is not understood. It can be suggested that one or multiple modes of action may be involved. These may include: (a) mutagenic and genotoxic events (e.g., direct and indirect effects on DNA and effects on chromosomes) by organic compounds in the gaseous and particle

phases: (b) indirect DNA damage via the production of reactive oxygen species (ROS) induced by particle-associated organics; and (c) particle-induced chronic inflammatory response leading to oxidative DNA damage through the release of cytokines, ROS, etc., and an increase in cell proliferation.

The particulate phase or whole DE exposure, as measured by DPM, appears to have the greatest observable contribution to the carcinogenic effects, and both the particle core and the associated organic compounds have demonstrated carcinogenic properties, although a role for the gas-phase components cannot be ruled out. The carcinogenic activity of DE may also be related to the small size of the particles. Moreover, the relative contribution of the possible mode(s) of action may be different at different exposure levels. For example, available evidence from rat studies indicates the importance of the role of the DPM in mediating lung tumor response at high exposure levels. Thus, the role of the adsorbed organic compounds may take on increasing importance at lower exposure levels.

7.5.5. Characterization of Overall Weight of Evidence: EPA's 1986 Guidelines for Carcinogen Risk Assessment

The totality of evidence supports the conclusion that DE is a *probable human carcinogen (Group B1)*. This conclusion is based on:

- "Limited"evidence (i.e., strong but less than sufficient evidence for "known human carcinogen"), for a causal association between DE exposure and increased risk of lung cancer among workers in different occupations;
- Evidence of carcinogenicity of DPM in rats and mice by noninhalation routes of exposure (intratracheal instillation, lung implantation, skin painting, and subcutaneous injection); and
- Extensive supporting data including the demonstrated mutagenic and/or chromosomal effects of DE and its organic constituents, suggestive evidence for the bioavailability of the organics from DE, and knowledge of the known mutagenic and/or carcinogenic activity of a number of individual organic compounds present on the particles (e.g,. PAH and derivatives) and in the DE gases (e.g., benzene, 1,3-butadiene, and aldehydes).

7.5.6. Weight-of-Evidence Hazard Narrative: EPA's Proposed Guidelines for Carcinogen Risk Assessment (1996b, 1999)

The combined evidence supports the conclusion that DE is *likely to be carcinogenic to humans* by inhalation and that this hazard applies to environmental exposure conditions. The spectrum of evidence and the inferences drawn provide a substantial case for this hazard potential. The weight of evidence of human carcinogenicity is based on:

- Strong but less than sufficient epidemiologic evidence for a causal association between DE exposure and increased risk of lung cancer among workers in different occupations;
- Evidence of carcinogenicity of DPM in rats and mice by noninhalation routes of exposure (intratracheal instillation, lung implantation, skin painting, and subcutaneous injection); and
- Extensive supporting data including the demonstrated mutagenic and/or chromosomal effects of DE and its organic constituents, suggestive evidence for the local and systemic bioavailability of the organics from DE, and knowledge of the known mutagenic and/or carcinogenic activity of a number of individual organic compounds present on the particles (e.g., PAH and derivatives) and in the DE gases (e.g., benzene, 1,3 butadiene, and aldehydes).

The weight-of-evidence for the lung cancer hazard is considered strong, even though inferences and uncertainties are involved. Major uncertainties include:

- There is scientific debate about the significance of the occupational epidemiologic evidence for a causal association between occupational exposure and increased lung cancer risk. Some experts view the evidence as weak given that most of the relative risk increases are <2.0, whereas others consider the evidence as more than adequate and compelling. With relatively low relative risks (<2.0), the effects of possible confounding exposures or other factors could play a significant role in the risk increases. For example, there is specific concern about whether the effects of smoking, a known cause of lung cancer, has been adequately or fully accounted for in the key studies. In more general terms, the lack of historical exposure data to retrospectively validate estimated DE exposure levels is also a limitation.
- A lack of knowledge about the mode(s) of action of DE lung cancer in humans results in the use of a number of default risk assessment assumptions which, while justifiable by evidence or policy choice, introduce uncertainty. To date, available evidence for the role of DPM, both the adsorbed organics and the carbon core

particle, has been shown only for high exposure conditions in the rat lung. The tumor inducing mode-of-action in the rat lung appears to depend on particle overloading of the lung and subsequent pathology. This sequence is judged not to be relevant for assessing the hazard to humans exposed in the ambient environment. There is virtually no information about the relative role of DE constituents in mediating the carcinogenic effects at lower experimental exposure levels, though hypotheses exist.

While a major uncertainty relates to the incomplete understanding of DE's mode(s) of action for the induction of lung cancer in humans, available data and hypotheses suggest that DE-induced lung carcinogenicity may be mediated by mutagenic and nonmutagenic events from both the particles and the associated organic compounds, although a role for the organics in the gaseous phase cannot be ruled out. Given that there is some evidence for a mutagenic mode of action, a cancer hazard is presumed at environmental exposure levels. This is consistent with EPA's science policy position, which assumes a nonthreshold effect for carcinogens in the absence of definitive data demonstrating a nonlinear or threshold mechanism. It should also be noted that there are not orders of magnitude differences between lower level occupational and higher end environmental exposure levels, in fact, there appears to be exposure overlap. This observation means that an extrapolation of the occupational hazard to lower environmental exposure levels is minimal, and thus, the conclusion of an environmental hazard is supported. Given these circumstances, linear low-dose extrapolation also would be an appropriate default choice in dose-response assessment that is focused on environmental levels of exposure (Chapter 8, Section 8.2). Because of insufficient information, the human carcinogenic potential of DE by oral and dermal exposures cannot be determined.

7.6. EVALUATIONS BY OTHER ORGANIZATIONS

Several organizations have reviewed the relevant data and evaluated the potential human carcinogenicity of DE or its particulate component. The conclusions reached by these organizations are generally comparable to the evaluation made in this assessment using EPA's Carcinogen Risk Assessment Guidelines. A summary of available evaluations conducted by other organizations is provided in Table 7-9.

7.7. CONCLUSION

It is concluded that environmental exposure to DE may present a lung cancer hazard to humans. The particulate phase appears to have the greatest contribution to the carcinogenic effect, both the particle core and the associated organic compounds have demonstrated

Table 7-9. Evaluations of DE as to human carcinogenic potential

Organization	Human data	Animal data	Overall evaluation
NIOSH (1988)	Limited	Confirmatory	Potential occupational carcinogen
IARC (1989)	Limited	Sufficient	Probably carcinogenic to humans
IPCS (1996)	N/A[a]	N/A	Probably carcinogenic to humans
California EPA (1998)	"Consistent evidence for a causal association"	"Demonstrated carcinogenicity"	DPM as a "toxic air contaminant" (California Air Resources Board)
NTP (2000)	"Elevated lung cancer in occupationally exposed groups"	"Supporting animal and mechanistic data"	DPM-Reasonably anticipated to be a carcinogen

[a]Not applicable.

carcinogenic properties, although a role for the DE gas-phase components cannot be ruled out. Using either EPA's 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) or the proposed revisions (U.S. EPA, 1996b, 1999), DE is judged to be a probable human carcinogen, or likely to be carcinogenic to humans by inhalation, respectively. The weight of evidence for potential human carcinogenicity for DE is considered strong, even though inferences are involved in the overall assessment. Major uncertainties of the hazard assessment include the following unresolved issues:

- There has been a considerable scientific debate about the significance of the available human evidence for a causal association between occupational exposure and increased lung cancer risk. Some experts view the evidence as weak given that most of the relative risk increases are <2.0 whereas others consider the evidence as more than adequate and compelling. Additionally, there is debate about whether the effects of smoking have been adequately accounted for in key studies, as well as the lack of historical DE exposure data to retrospectively validate estimated DE exposure levels for the available studies.
- A lack of knowledge about the mode(s) of action for DE lung cancer in humans results in the use of a number of default risk assessment assumptions which, while justifiable by evidence or policy choice, introduce uncertainty. To date, available evidence for the role of DPM, both the adsorbed organics and the carbon core particle, has been shown only for high-exposure conditions in the rat lung. The

tumor-inducing mode of action in the rat lung appears to depend on particle overloading of the lung but this is judged to be not relevant for assessing the human hazard of ambient exposures. There is virtually no information about the relative role of DE constituents in mediating carcinogenic effects at lower experimental or environmental exposure levels. Furthermore, there is only a limited understanding regarding the relationship between DPM particle size and carcinogenicity.

- DE is present in ambient PM (e.g., $PM_{2.5}$ or PM_{10}); however, a cancer hazard for ambient PM has not been identified, as of 1996 (EPA 1996b). An updated evaluation is expected in 2002.

Additional research is needed to address these issues to reduce the uncertainty associated with the potential cancer hazard of exposure to DE.

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8. DOSE-RESPONSE ASSESSMENT: CARCINOGENIC EFFECTS

8.1. INTRODUCTION

Dose-response assessment for carcinogenicity defines the relationship between the exposure/dose of an agent and the degree of carcinogenic response, and evaluates potential cancer risks to humans at exposure/dose levels of interest. Most often, the exposure/dose response of interest is well below the range of observation. As a result, dose-response assessment usually entails an extrapolation from the generally high exposures in studies on humans or laboratory animals to the exposure levels expected from human contact with the agent in the environment. It also includes considerations of the scientific validity of these extrapolations based on available knowledge about the underlying mechanisms or modes of carcinogenic action. The complete sequence of biological events that must occur to produce an adverse effect is defined as "mechanism of action." In cases where only partial information is available, the term "mode of action" is used to refer to the mechanisms for key events that are judged to be sufficient to inform about the shape of the dose-response curve beyond the range of observation.

This chapter evaluates the available exposure/dose-response data and discusses extrapolation issues in estimating the cancer risk of environmental exposure to diesel engine exhaust (DE). It concludes that available data are inadequate to confidently derive a cancer unit risk estimate for DE or its component, diesel particulate matter (DPM). Unit risk is one possible output from a dose-response assessment and is defined as the estimated upper-bound cancer risk at a specific exposure or dose from a continuous average lifetime exposure to a carcinogen (in this case, cancer risk per $\mu g/m^3$ of DPM). In lieu of unit-risk-based quantitative risk estimates, this chapter provides a perspective about potential risk at environmental levels. Subsequent sections of this chapter discuss issues related to dose-response evaluation of human cancer risk for DE exposure, including the target tumor site and underlying mode of action, suitable measures of dose, approaches to low-dose extrapolation, and appropriate data to be used in the dose-response analysis. This is followed by a simple analysis of the possible degree and extent of risk from environmental exposure to DE.

Appendix C provides a summary review of dose-response assessments conducted to date by other organizations and investigators. These risk estimations were performed on the basis of either epidemiologic and/or experimental data. As concluded in Section 8.5, EPA finds that available epidemiologic data are too uncertain to confidently derive a unit risk estimate for DE-induced lung cancer, and that rat data are not suitable for estimating human risk. Nevertheless, a review of dose-response evaluations is provided in the appendix for historical context.

8.2. MODE OF ACTION AND DOSE-RESPONSE APPROACH

According to EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996), dose-response assessment is performed in two steps: assessment of observed data to derive a point of departure, followed by extrapolation to lower exposures to the extent necessary. Human data are always preferred over animal data, if available, as their use obviates the need for extrapolation across species. Mode-of-action information is important to dose-response evaluation, as it informs about the relevance of animal data to assessment of human hazard and risk, the shape of the dose-response curve at low doses, and the most appropriate measure(s) of exposure/dose and response.

If there are sufficient quantitative data (humans and/or animals) and adequate understanding of the carcinogenic process, the preferred approach is to use a biologically based model for both the range of observation and extrapolation below that range. Otherwise, as a default procedure, a standard mathematical model is used to curve-fit the observed dose-response data to obtain a point of departure, which is the lower 95% confidence limit of the lowest exposure/dose that is associated with a selected magnitude of excesses of cancer risk in human or animal studies. Default approaches for low-dose extrapolation should be consistent with the current understanding of the mode(s) of action. These include approaches that assume linearity or nonlinearity, or both. Linear extrapolation is used when there is insufficient understanding of the modes of action, or the mode-of-action information indicates that the dose-response curve at low dose is, or is expected to be, linear. Linear extrapolation involves the calculation of the slope of the line drawn from the point of departure to zero exposure or dose (i.e., above background). When there is sufficient evidence for a nonlinear mode of action but not enough data to construct a biologically based model for the relationship, a margin of exposure is used as a default approach. A margin-of-exposure analysis compares the point of departure (i.e., the lowest exposure associated with some cancer risk) with the dose associated with the environmental exposure(s) of interest and determines whether or not the exposure margins are adequate. Both default approaches may be used for a tumor response if it is mediated by linear and nonlinear modes of action. The dose-response approaches considered in this chapter follow the principles of EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986, 1996, 1999).

As reviewed in Chapter 7, there is substantial evidence from combined human and experimental evidence that DE is likely to pose a cancer hazard to humans at anticipated levels of environmental exposure. The critical target organ is the lung. Evidence exists for a causal relationship between risk for lung cancer and occupational exposure to DE in certain occupational workers such as railroad workers, truck drivers, heavy equipment operators, transit workers, etc.

The mechanism(s) by which DE induces lung cancer in humans has not been established. As discussed in Chapter 7, Section 7.4, several modes of action have been postulated on the basis of available mechanistic studies, including direct DNA effects (gene mutations) by the adsorbed organic compounds and the gaseous fractions, indirect DNA effects (e.g., chromosomal aberrations, sister chromatid exchange [SCE], micronuclei) by DE and DPM, oxidative DNA damage by DPM via release of reactive oxygen species (ROS), and particle-induced chronic inflammatory response leading to epithelial cell cytotoxicity and regenerative cell proliferation via release of cytokines, growth factors, and ROS. It is likely that a combination of modes of action contributes to the overall carcinogenic activity of DE, and that the relative contribution of the various modes of action may vary with different exposure levels.

In the absence of a full understanding of the relative roles of DE constituents in inducing lung cancer in humans, and because there is some evidence for a mutagenic mode of action, linear low-dose extrapolation is an appropriate and prudent default choice for modeling dose-response, and if needed, risk extrapolation from high to lower exposures (U.S. EPA, 1986, 1996, 1999). It also should be noted that there are not order of magnitude differences between lower levels of occupational and higher end environmental exposure estimates. In fact, there appears to be exposure overlap. This means that an extrapolation of the occupational hazard to lower environmental exposure levels is minimal. Other individuals and organizations have used either linear risk extrapolation models and/or mechanistically based models to estimate cancer risk from environmental exposure to DE (e.g., IPCS, 1996; Cal EPA, 1998; also see Appendix C). These were examined but not found to provide a compelling basis for unit risk derivation because of database uncertainty and/or recent understandings about the suitability of the rat data for modeling a dose-response at environmental levels of exposure.

For example, there are an observable series of events showing how DE causes lung tumors in the rat under high exposure experimental conditions. Prolonged exposure to high concentrations of a variety of poorly soluble particles including DPM (and its carbon core, devoid of organics) causes lung tumors in rats through a mode of action that involves impairment of lung clearance mechanisms (referred to as "lung overload response"), leading to persistent chronic inflammation, cell proliferation, metaplasia, and ultimately the development of lung tumors (ILSI, 2000). Because this mode of action is not expected to be operative at environmental exposure conditions, the rat lung tumor dose-response data are not considered suitable for predicting human risk at low environmental exposure concentrations.

8.3. USE OF EPIDEMIOLOGIC STUDIES FOR QUANTITATIVE RISK ASSESSMENT

As discussed above, human data are considered more appropriate than animal data in estimating environmental cancer risk for DE. Still, there are many uncertainties in using the

available epidemiologic studies that have quantitative exposure data to extrapolate the risk to the general population for ambient-level DE exposure.

8.3.1. Sources of Uncertainty

The greatest uncertainty in estimating DE-induced cancer risk from epidemiologic studies is the lack of knowledge of actual historical exposures for individual workers, particularly for the early years. Reconstruction of historic exposures is based on job exposure categories, industrial hygiene measurements, and assumptions made about exposure patterns.

Another related uncertainty is the choice of markers of exposure to DE. As discussed above, the modes of action for DE-induced lung cancer in humans are not fully understood, and thus the best measure of DE exposure is unknown. Various markers of DPM (e.g., respirable-sized particles, elemental carbon [EC]) have been used as dosimeters for DE. Though EC is more sensitive and more specific than respirable-sized particles, both are considered appropriate dosimeters. Related to the choice of dosimeter, having a relatively constant relationship between the organics (on the particle) and the particle mass would be consistent with a possible mode-of-action role for both the particle and organic components. However, evidence of such a constant historic relationship remains unclear. As discussed in Chapter 2 (Section 2.5.2), it appears that newer model on-road engine exhaust has a lesser quantity of organics adsorbed onto the particle compared to older model engines. On the other hand, with regard to DE in the ambient air, there is significant variation in the amounts of DPM organic components emitted because of aged vehicles in the on-road fleet, driving patterns, and the additional presence of nonroad DE (e.g., marine vessels and locomotives, which generally use older technology than on-road engines).

Another major uncertainty associated with many of the DE epidemiologic studies was the inability to fully control for smoking effects, resulting in possible errors in estimating relative risk increases. Changes in adjustments for smoking could result in considerable changes in relative risk, because smoking has a much larger effect on relative lung cancer risk than is likely for DE. It is difficult to effectively control for a smoking effect in a statistical analysis because cigarette smoke contains an array of biologically active compounds and affects multiple steps of carcinogenesis, thus probably making smokers more susceptible to DE-induced lung cancer than are nonsmokers. A statistical analysis would not be able to adjust for such an effect without having a detailed record of the smoking history of individuals.

A potential uncertainty involves the use of occupational worker data to extrapolate cancer hazard risk to the general population and sensitive subgroups. By sex, age, and general health status, workers are not fully representative of the general population. For example, there is virtually no information to determine whether infants and children or people in poor health

respond differently to DE exposure than do workers. Finally, the use of linear low-dose extrapolation may contribute to uncertainty in estimating environmental risks.

8.3.2. Evaluation of Key Epidemiologic Studies for Potential Use in Quantitative Risk Estimates

Among the available epidemiologic studies, only the railroad worker studies and the Teamster truck driver studies have reconstructed quantitative historical exposure data for possible use in deriving a unit risk estimate for DE-induced lung cancer. This section evaluates the strengths and limitations of these data and their suitability for dose-response analysis.

8.3.2.1. *Railroad Worker Studies*

Garshick and colleagues conducted both cohort and case-control studies of lung cancer mortalities among U.S. railroad workers registered with the U.S. Railroad Retirement Board (RRB).

In the cohort study (Garshick et al., 1988), lung cancer mortality was ascertained through 1980 in 55,407 railroad workers, age 40 through 64 in 1959, with at least 10 years of work in selected railroad jobs (39 job titles). The cohort was selected on the basis of job titles in 1959. Industrial hygiene evaluations and descriptions of job activities were used to classify jobs as exposed or unexposed to diesel emissions. Workers with recognized asbestos exposure were excluded from the job categories selected for study. However, a few jobs with some potential for asbestos exposure were included in the cohort. 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 DE exposure for this study because by this time 95% of the locomotives in the United States were diesel powered. The author reported statistically significant relative risk increases of 1.57 for the 40-44 year age group and 1.34 for the 45-49 year age group, after exclusion of workers exposed to asbestos and controls for smoking. Age groups were determined by their ages in 1959.

A main strength of the cohort study is the large sample size (55,407), which allowed sufficient power to detect small risks. This study 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 64 years of age in 1959 classified as DE-exposed, 94% of the cases were still in DE-exposed jobs 20 years later.

The main limitation of the cohort study is the lack of quantitative data on exposure to DE. The number of years exposed to DE was used as a surrogate for dose. The dose, based on duration of employment, has inaccuracies because individuals were working on both steam and diesel locomotives during the transition period. It should be noted that the investigators included

only exposures after 1959; the duration of exposure prior to 1959 was not known. Other limitations of this study include its inability to examine the effect of years of exposure prior to 1959 and the less-than-optimal latency period for lung cancer expression. No adjustment for smoking was made in this study. For a detailed description of this study please refer to Chapter 7, Section 7.2.1.7.

Garshick and colleagues also conducted a case-control study of railroad workers who died of lung cancer between 1981 and 1982 (Garshick et al., 1987). The author reported statistically significant increased odds ratios (with asbestos exposure accounted for) of 1.41 (95% confidence interval [CI] = 1.06, 1.88) for the ≤64 year age group and 1.64 (95% CI = 1.18, 2.29) for the ≤64 year age group with ≥20 years of exposure when compared with the 0-4 year exposure group. 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 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 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. A total of 1,256 cases and 2,385 controls were selected for the study. Among younger workers, approximately 60% had exposure to DE, whereas among older workers, only 47% were exposed to DE. DE exposure surrogates for workers were similar to those in the cohort study. Asbestos exposure was categorized on the basis of jobs held in 1959, or on the last job held if the subject retired before 1959. Smoking history information was obtained from the next of kin.

The strengths of the case-control study are consideration of confounding factors such as asbestos exposure and smoking; classification of DE exposures by job titles and industrial hygiene sampling; and exploration of interactions between smoking, asbestos exposure, and DE exposure. Major limitations of this study include (a) possible overestimation of cigarette consumption by surrogate respondents; (b) use of the Interstate Commerce Commission (ICC) job classification as a surrogate for exposure, which may have led to misclassification of DE exposure jobs with low intensity and intermittent exposure, such as railroad police and bus drivers, as unexposed; (c) lack of data on the contribution of unknown occupational or environmental exposures and passive smoking; and (d) a suboptimal latency period of 22 years, which may not be long enough to observe a full expression of lung cancer. For a detailed description of this study, please see Chapter 7, Section 7.2.2.4.

As a part of these epidemiologic studies, Woskie et al. (1988a) conducted an industrial hygiene survey in the early 1990s for selected jobs in four small northern railroads. DE

exposure was considered as a yes/no variable based on job in 1959 and estimated years of work in a diesel- exposed job as an index of exposure. Thirty-nine job titles were originally identified and were then collapsed into 13 job categories and, for some statistical analyses, into 5 categories (clerks, signal maintainers, engineers/firers, brakers/conductors/hostlers, and shop workers) (Woskie et al., 1988b; Hammond et al., 1988). As discussed below, these exposure estimations were used by Crump et al. (1991) and by Cal EPA (1998) for their dose-response analyses.

8.3.2.1.1. ***Potential for the data to be used for dose-response modeling.*** Both case-control and cohort studies can be used for dose-response analysis if exposure for each worker is available. Control of a smoking effect is important when lung cancer is the disease of interest. However, as discussed previously (see Section 8.3.1), one may not be able to control smoking completely in a dose-response analysis because of the lack of detailed records of the smoking history of individuals.

Garshick et al. (1988) reported a positive relationship of relative risk and duration of exposure by modeling age in 1959 as a covariate in an exposure-response analysis. The positive relationship disappeared when attained age was used instead of age in 1959, and a negative dose-response was observed (Crump et al., 1991). This negative dose-response continued to be upheld in a subsequent reanalysis (Crump, 1999). Garshick (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991) performed further analysis and reported that the relationship between years of exposure and risk of lung cancer, when adjusted for attained age and calendar year, was flat to negative depending upon which model was used. In contrast, California EPA (Cal EPA, 1998) found a positive dose-response by using age in 1959 but allowing for an interaction term of age and calendar year in the model.

Crump et al. (1991) also found, and Garshick (letter from Garshick, Harvard Medical School, to Chao Chen, U.S. EPA, dated August 15, 1991) confirmed, that in the years 1977-1980 the death ascertainment was not complete. About 20% to 70% of deaths were unaccounted for, depending upon the calendar year. Further analysis, based on job titles in 1959 and limited to deaths occurring through 1976, showed that the youngest workers still had the highest risk of dying of lung cancer.

Extensive statistical analyses were conducted by a panel convened by HEI (1999) to investigate the utility of the railroad worker cohort for use in dose-response based quantitative risk assessment. Seven models were used to test the data, and the models were formed by varying a number of covariates in different combinations. The covariates included employment duration, cumulative exposure with and without correction for background exposure, and three job categories: clerks and signalmen, train workers (which include engineers/firers/brakers/

conductors), and shop workers. The coefficient for each covariate in a model is used to calculate relative risk for the associated covariate. In summary, the panel found that effects of exposure as defined by an exposure-response curve were either flat or negative in all of the models. In these analyses, relative risk for each job category was assumed to be constant with respect to age. Further exploration of the data showed that the relative risk for train workers was not constant. The panel's statistical analyses also revealed the complexity of the data and difficulties of providing an adequate summary measure of effect, probably because calendar year and cumulative exposure are highly correlated, which makes it especially difficult to sort out their separate effects. The difficulty of providing an adequate measure of DE effect was further demonstrated in Table C.3 of the HEI report, in which negative or positive effects for cumulative exposure (with background exposure adjustment) were obtained depending on whether or not job category was included in the model. A similar review of the divergent views about the railroad worker dose response also can be found in Chapter 7, Section 7.2.1.7.

The diverging results about the presence or absence of exposure response for the railroad worker data have become a source of continuing debate about the suitability of these data for estimating DE cancer risk. Although it is difficult to identify the exact reason for the diverging findings, the "age effect" appears to be a main source of uncertainty because age, calendar year, and cumulative exposure are not mutually independent. Therefore, an ideal dose-response analysis would account for the ages when exposure to DE began and terminated, along with the attained age and other covariates for each person, using age-dependent exposure concentration rather than cumulative exposure over lifetime as a dosimeter. This analysis would be possible for the railroad workers if information were available on the ages when exposure began and terminated.

Given the equivocal evidence for positive exposure response, EPA has not derived a unit risk on the basis of the available railroad worker data. This determination should not be construed, however, to imply that the railroad worker studies contain no useful information on lung cancer risk from exposure to DE.

8.3.2.2. *Teamsters Union Trucking Industry Studies*

Steenland et al. (1990) conducted a case-control study of lung cancer deaths in the Central States Teamsters Union to determine the risk of lung cancer among different trucking industry occupations. The study found statistically significant increased odds ratios for lung cancer of 1.89 and 1.64, depending on years of employment. Cases comprised all deaths from lung cancer (1,288). The 1,452 controls comprised every sixth death from the entire file, excluding deaths from lung cancer, bladder cancer, and motor vehicle accidents. Individuals were required to have 20 years tenure in the union to be eligible to claim benefits.

Detailed information on work history and potential confounders such as smoking, diet, and asbestos exposure was obtained by questionnaire. On the basis of interview data and the 1980 census occupation and industry codes, subjects were classified either as nonexposed or as having held other jobs with potential DE exposure. The 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 main strengths of the study are the availability of detailed records from the Teamsters Union, a relatively large sample size, availability of smoking data, and measurement of possible asbestos exposures. Some limitations of this study include possible misclassifications of exposure and smoking habits, as information was provided by next-of-kin and lack of sufficient latency to observe lung cancer excess.

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 with exposure estimates based on a 1990 industrial hygiene survey of EC exposure (Zaebst et al., 1991), a surrogate for DE in the trucking industry. Available data indicate that exposure to workers in the trucking industry in 1990 averaged 2-27 $\mu g/m^3$ of EC. The 1990 exposure information was used by Steenland 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.

The industrial hygiene survey by Zaebst et al. (1991) of EC exposures in the trucking industry provided exposure estimates for each job category in 1990. The EC measurements were generally consistent with the epidemiologic results, in that mechanics were found to have the highest exposures and relative risk, followed by long-haul and short-haul drivers. Dockworkers who had the lowest exposures also had the lowest relative risks.

Past exposures were estimated assuming that they were a function of (a) the number of heavy-duty trucks on the road, (b) the particulate emissions (grams/mile) of diesel engines over time, and (c) leaks from truck exhaust systems for long-haul drivers. Estimates of past exposure to EC (as a marker for DE exposure) were made based on the assumption that average 1990 levels for a particular 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. For example, a 1975 exposure level 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. As with most epidemiologic studies, the endeavors to reconstruct exposures for epidemiologic studies are subject to uncertainties.

8.3.2.2.1. ***Potential for the data to be used for dose-response modeling.*** Steenland et al. (1998) analyzed their case-control data and showed a significant positive trend in lung cancer risk with increasing cumulative exposure to DE. The study by Steenland et al. (1998) could provide a valuable database for calculating unit risk for DE emissions. The strength of this data set is that the smoking histories 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 than does DE. In the Steenland et al. (1998) study, the overall (ever-smokers vs. nonsmokers) odds ratio for developing lung cancer from smoking is about 7.2, which is about fivefold larger than the 1.4 relative risk increase from a large synthesis of many DE epidemiologic studies. It is possible that a modest change of information on smoking and diesel exposure might alter the conclusion and risk estimate.

Another strength of the Teamster data for use in environmental risk assessment for the general population is that exposures of Teamsters are closer to ambient exposures than are those of railroad workers. The Teamsters Union truck driver case-control workers had cumulative exposure ranging from 19 to 2,440 $\mu g/m^3$-years of EC, with the median and 95th percentile, respectively, of 358 and 754 $\mu g/m^3$-years of EC. The median and 95th percentile of an environmentally equivalent exposure would be 3 and 6 $\mu g/m^3$, respectively.[1] These environmental equivalent exposures for the Teamsters Union truck drivers are close to the estimated ambient exposures of <1.0 $\mu g/m^3$ to 4.0 $\mu g/m^3$ (see Table 2-31).

Steenland et al. (1998) stated that their risk assessment is exploratory because it depends on estimates about unknown past exposures. An EPA reanalysis of DE exposure for this study is underway. With a revised exposure assessment, a reevaluation of the dose-response would be appropriate. In a recent review, HEI (1999) concluded that the Teamsters studies may be useful for quantitative risk assessment, but significant further evaluation and development are needed. Given the ongoing reanalysis of exposure, EPA will not, at this time, use the Steenland et al. (1998) occupational risk assessment findings to derive equivalent environmental parameters and cancer unit risk estimates.

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[1]The conversion assumes (a) DPM = 40% EC as reported by Steenland et al. (1998), (b) environmental equivalent exposure is approximately = 0.21 × occupational exposure, and (c) 70 $\mu g/m^3$-years is equivalent to a lifetime of exposure at 1 $\mu g/m^3$.

8.3.3. Conclusion

Even though available evidence supports a conclusion that DE is likely to be a human lung carcinogen, the conclusion of the dose-response evaluation is that the available data are not sufficient to confidently estimate a cancer unit risk or unit risk range. The absence of such a cancer unit risk for DE limits the ability to quantify, with confidence, the potential impact of the hazard on exposed populations. Two significant short-term activities are underway to improve the epidemiologic database for dose-response assessment: (1) a follow-up study to correct the undercounting of mortality in the Garshick et al. (1988) railroad worker study, and (2) an EPA-sponsored effort to improve the exposure estimates for Teamsters Union truck drivers (Steenland et al., 1998). EPA will monitor this ongoing research as well as the ongoing NCI-NIOSH study of nonmetal miners and the recently NCI-funded epidemiologic study of truck drivers. As these studies or other new data become publicly available, EPA will reconsider the merit of conducting additional dose-response analysis and unit risk derivation.

8.4. PERSPECTIVES ON CANCER RISK

Although the available data are considered inadequate to confidently estimate a cancer unit risk, this does not mean that there is no information about the possible cancer risk of DE. To examine the significance of the potential cancer hazard from environmental exposure to DE, all relevant epidemiologic and exposure data as well as simple risk assessment tools can be used. Such an approach does not produce confident estimates of cancer unit risk. Rather, these exploratory approaches provide a perspective on the possible magnitude of cancer risk and thus insight about the potential significance of the hazard. This section describes approaches and methods that are used to gauge the magnitude of possible cancer risk from ambient exposure to DE.

The first approach involves examining the differences between the levels of occupational and ambient environmental exposures, while assuming that cancer risk to DE is linearly proportional with cumulative lifetime exposure. Risks to the general public would be low in comparison to occupational risk if the differences in exposure are large (e.g., about three orders of magnitude or more). On the other hand, if the exposure differences are smaller (i.e., within one to two orders of magnitude), environmental risks become more of a concern as they approach the range of workers' risk observed in epidemiologic studies of past occupational exposures. This assumes that the carcinogenic potency of historical and current-day DE is not significantly different, a reasonable assumption, though not without uncertainty.

Table 8-1 shows occupational exposure estimates for some of the occupational groups where increased relative risks of lung cancer (e.g., meta-analyses) have been analyzed. Given that no statistical properties associated with these exposure estimates are known, their use here is

Table 8-1. DPM exposure margins (ratio of occupational ÷ environmental exposures)

Occupational group	Estimated occupational exposure/concentration ——————— Environmental equivalent [a]	Exposure margin ratio - average environmental exposure for 0.8 $\mu g/m^3$ of environmental exposure[b]	Exposure margin ratio - high-end environmental exposure for 4.0 $\mu g/m^3$ of environmental exposure[b]	Reference[c]
Public transit workers	15-98 $\mu g/m^3$ ---------- 3-21 $\mu g/m^3$	4-26	0.8-5	Birch and Cary, 1996
U.S. railroad workers	39-191 $\mu g/m^3$ 8- 40 $\mu g/m^3$	10-50	2-10	Woskie et al., 1988b
Fork Lift Operators	7-403 $\mu g/m^3$ ---------- 1- 85 $\mu g/m^3$	2-106	0.37-21	Groves and Cain, 2000[d]
High end boundary estimate	1200 $\mu g/m^3$ ---------- 252 $\mu g/m^3$	315	63	see text discussion in Section 8.4

[a] Equivalent environmental exposure = occupational exposure × 0.21, see Chapter 2, Section 2.4.3.1, some values are rounded.
[b] 0.8 $\mu g/m^3$ = average 1990 nationwide on-road exposure estimate from HAPEM model; the companion rural estimate is 0.5 $\mu g/m^3$, and 4 $\mu g/m^3$ is
a high-end estimate. The 1996 nationwide average is 0.7 $\mu g/m^3$, the companion rural estimate is 0.2 $\mu g/m^3$; however, a high-end estimate is not available for 1996. See Chapter 2, Sections 2.4.3.2.1 and 2.4.3.2.2.
[c] See Table 2-27 for more details about Birch and Cary, Woskie.
[d] 403 $\mu g/m^3$ is a 99 percentile estimate of EC/$\mu g/m^3$, the DPM equivalent of the EC measurement can be estimated as DPM = EC x 0.62 to 1.31.

not intended to be precise or to match with specific epidemiologic data, but rather to provide a broad range of possible exposures in the workplace. The purpose is to identify a high- and low-end occupational exposure consistent with the occupational groups of interest and then to compare these to estimates of environmental exposure. Given the special interest in discerning the lower risk magnitude, especially to see if the lower risk might be above or below one in 1 million, a high-end exposure estimate would be used, and as discussed later, the occupational exposure can be arbitrarily increased (e.g., toward an extreme value) to ensure that a low end of risk is identified, consistent with the reported occupational risk increases. Environmental exposure data from on-road vehicle emissions are based on the 1990 nationwide exposure estimates from the HAPEM model (see Chapter 2, Section 2.4.3.2.1). Both average (0.8 $\mu g/m^3$) and high-end exposures (4 $\mu g/m^3$) are used.

In order to compare differences between occupational and environmental exposures, it is necessary to convert occupational exposure to continuous exposure (i.e., environmental equivalent exposure = 0.21 × occupational exposure, see Section 2.4.3.1). Accordingly, Table 8-1 shows equivalent environmental levels and the ratios of occupational to environmental

exposures, referred to as exposure margins (EMs). An EM of 1 or less indicates that environmental exposure is comparable to occupational exposure. An EM >1 means that the occupational equivalent exposure is greater than the benchmarked environmental exposure.

Table 8-1 shows that the EMs based on the average nationwide environmental exposure (0.8 $\mu g/m^3$) approach three orders of magnitude. EM's that range from 1 to 10 also can be viewed as showing that adjusted occupational exposures are relatively close to the ambient environmental levels that were chosen as benchmarks. This closeness sets the stage for less uncertainty in hazard and risk extrapolation from the occupational to environmental setting. It also raises a concern that risks to the general public could approach worker risks that were observed in the occupational epidemiologic studies. Table 8-1 is based upon DE exposure estimates from on-road sources only. With the addition of exposure from nonroad sources, the average nationwide-based EM ratios would be lower. For example, using 1996 exposure data for urban populations (Table 2-30), the exposure from on-road sources is 0.5 $\mu g/m^3$, whereas nonroad sources contribute 0.9 $\mu g/m^3$, for a total of 1.4 $\mu g/m^3$. Using this exposure value in place of the EM calculation of Table 8-1 (1990 estimate of 0.8) produces a nearly 43% reduction in the EM ratio. A comparison of EM changes for the high-end on-road plus nonroad exposure is not possible at the present time because the 1996 data have not yet been modeled to obtain a high-end value similar to the 1990 value of 4.0 $\mu g/m^3$.

A second approach to explore the possible cancer risk to the general population from environmental exposure to DE is more quantitative. One begins by examining the risk observed in DE-exposed workers and then making reasoned assumptions as to how these risks can be translated to environmental exposure conditions. Such an approach involves three major assumptions: (1) the excess lung cancer risk as shown in numerous epidemiologic studies and in two meta-analyses is indeed due to DE exposure, (2) the increased lung cancer risk over background is linearly proportional to the lifetime exposure to DE, and (3) the past DE exposure for workers has the same cancer-inducing potential as the current DE in ambient air. Any of these assumptions could have an impact on the possible environmental risk by either increasing or decreasing the risk estimates, including the possibility of a lower or zero risk at environmental levels.

As reviewed in Chapter 7, Section 7.2, numerous epidemiologic studies have shown increased lung cancer risks (i.e., some are deaths, some are cases) among workers in certain occupations. The relative risks or odds ratios range from 1.2 to 2.6. Two independent meta-analyses show smoking-adjusted relative risk increases of 1.35 (Bhatia et al., 1997) and 1.47 (Lipsett and Campleman, 1999). For this analysis, a relative risk of 1.4 is selected as a reasonable estimate. This risk means that the workers faced an extra risk 40% higher than the

5% background lifetime lung cancer risk in the U.S. population.[2] Thus, using the relationship [excess risk = (relative risk-1) × background risk], these DE-exposed workers would have an excess risk of 2% (10^{-2}) (i.e., to develop lung cancer) due to occupational exposure to DE [(1.4 - 1) × (0.05) = 0.02]. The validity of this interpretation depends on an important assumption: that the observed incremental risk of 40% was due to DE exposure alone and not to other unknown extraneous factors. It should be noted, however, that the conclusion about the risk perspective would not be changed even if the incremental risk of 40% were greatly reduced (e.g., to 20%); the conclusion would be changed only if almost all of the incremental risk were due to nondiesel factors.

Next, one would consider the EM (i.e., the EM ratio) between the occupational exposures and general-population environmental exposures. DPM concentrations in the workplace, used as a surrogate for worker exposure, are shown for three occupational worker groups in Table 8-1. These range from 7-403 $\mu g/m^3$ (with an equivalent continuous exposure of 1-85 $\mu g/m^3$). These worker groups are consistent with many of those cited in the meta-analyses. For this exploratory risk estimation approach, we want to intentionally adopt a high-end boundary exposure estimate that is unlikely to be exceeded, so that a lower bounding of the risk would be identified. An occupational boundary exposure of 1,200 $\mu g/m^3$ with its environmental equivalent estimated value of 252 $\mu g/m^3$ has been purposefully adopted to represent a high-end boundary estimate. This happens to be about three times the forklift operator value shown in Table 8-1, and clearly a high-end estimate when Table 2-27 is examined, exclusive of the estimates for miners which are not included in the meta-analyses. It should be noted once again that none of these estimates are intended to be precise.

Table 8-1 shows that the DPM exposure margin ratio between occupational and environmental exposure, using the nationwide average exposure value of 0.8 $\mu g/m^3$, may range from 2 to 315 when the boundary estimate is used, and range from 0.37-63 when 4.0 $\mu g/m^3$ is used as a high-end environmental benchmark exposure. Some of these extreme values will be used, as discussed in the next paragraph.

Risks from environmental exposure depend on the shape of the dose-response curve in the range between occupational and environmental exposures. If lifetime risks in this range were

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[2]The background rate of 0.05 is an approximated lifetime risk calculated by the method of lifetable analysis using age-specific lung cancer mortality data and probability of death in the age group taken from the National Health Statistics (HRS) monographs of Vital Statistics of the U.S. (Vol. 2, Part A, 1992). Similar values based on two rather crude approaches also can be obtained: (1) $59.8 \times 10^{-5} / 8.8 \times 10^{-3} = 6.8 \times 10^{-2}$ where 59.8×10^{-5} and 8.8×10^{-3} are, respectively, the crude estimates of lung cancer deaths (including intrathoracic organs, estimated to be less than 105 of the total cases) and total deaths for 1996 reported in Statistical Abstract of the U.S. (Bureau of the Census, 1998, 118th Edition), and (2) 156,900/270,000,000 × 76 = 0.045, where 156,900 is the projected lung cancer deaths for the year 2000 as reported in Cancer Statistics J of the American Cancer Society, Jan/Feb 2000), 270,000,000 is the current U.S. population, and 76 is the expected lifespan.

to fall proportionately with reduced exposure, and if one assumes that past occupational exposures were at the high-boundary end, then the risk from average environmental exposure could be between 10^{-5} and 10^{-4} ($0.02 \div 315 = 6 \times 10^{-5}$). On the other hand, if occupational exposures for different groups were lower, risks from environmental exposure would be higher. For example, if occupational concentrations or exposures were closer to 100 $\mu g/m^3$, a value that is represented in several data sets shown in Table 8-1 (with an equivalent environmental exposure of 21 $\mu g/m^3$ and a corresponding EM of 26), then risks from environmental exposure would approach 10^{-3} ($0.02 \div 26 = 8 \times 10^{-4}$). If lifetime risks were to fall more than proportionately, then risks would be lower. The latter two sources of dose-response uncertainty (i.e., the actual occupational exposures and the shape of the dose-response curve at low exposures) cannot be further defined with currently available information. Use of higher environmental exposures (>0.8 up to 4.0 $\mu g/m^3$) lowers the EM value and increases the estimated risk.

The magnitude of the estimated lifetime cancer risk derived from using a very high-end occupational-to-environmental exposure difference, establishes a reasonable basis to believe that the general population could face possible risks high enough to be of concern. This does not directly address the segments of the population that may be at highest risk: those who are additionally exposed to nonroad sources of DE and children who may be more sensitive to early-life DE exposure, if not in fact, a longer period of potential lifetime exposure.

The analyses presented above are not intended to be precise but are useful in gauging the possible risk range using simple risk exploration methods. Best scientific judgment guided the selection of assumptions and other elements of this analysis which are deemed reasonable and appropriate for identifying possible risks based on the information currently available. These analyses provide a sense of where an upper limit (or "upper bound") of the cancer risk may be. The simple methodologies used are generic and are valid for any increased relative risk data, however, they are not unique to the DE data. These analyses are subject to numerous uncertainties, particularly the lack of actual exposure information in the epidemiologic studies and uncertainties related to the three major underlying assumptions mentioned earlier. Any of these uncertainties could have an impact on the possible risk levels discussed above. Lower risks are possible and one cannot rule out zero risk. The risks could be zero because (a) some individuals within the population may have a high tolerance to exposure from DE and therefore not be susceptible to the cancer risk from environmental exposure, and (b) although evidence of this has not been seen, there could be a threshold of exposure below which there is no cancer risk.

The estimated possible risk ranges (10^{-5} to 10^{-3} as well as lower and zero risk) provide a perspective of the potential significance of the lung cancer hazard. This perspective should not

be viewed as a definitive quantitative characterization of risk. The development of risk estimates does not constitute endorsement of their validity as surrogates for cancer unit risk or their suitability for estimating numbers of cancer cases. Further research is needed to more accurately assess and characterize environmental cancer risks of DE.

8.5. SUMMARY AND DISCUSSION

There are numerous published quantitative dose-response assessments to estimate human cancer risk from exposure to DE that use epidemiologic and/or experimental animal data (see Appendix C). These dose-response assessments were considered but failed to present a fully sufficient basis for a confident derivation of a cancer unit risk. This is because of epidemiologic exposure-related database uncertainties and the recent understanding about the lack of relevance of the rat lung cancer response to environmentally exposed humans. The dose-response analysis in this chapter has focused on the feasibility of using the occupational epidemiologic data to develop dose-response relationships and extrapolating them to the presumably lower levels of environmental exposure. Typically, this would result in the derivation of an exposure/dose-specific cancer unit risk. In the absence of an understanding about the mode(s) of action for DE-induced lung cancer in humans, coupled with the consideration that DE contains many mutagenic and carcinogenic constituents, linear low-dose extrapolation is judged to be an appropriate default choice for dose-response analysis, should there be satisfactory data to perform such an analysis.

This chapter specifically evaluated the suitability of using the railroad worker studies (Garshick et al., 1987, 1988) and the Teamster Union truck driver studies (Steenland et al., 1990, 1998) for dose-response analysis. However, because of uncertainties about the exposure response for the railroad workers and exposure uncertainties for the truck drivers, a dose-response-based cancer unit risk estimate for DE is not developed from these data sets at this time.

In the absence of a unit risk to assess environmental cancer risk, some insight about the possible significance of the hazard can be drawn from the available epidemiologic data and exploratory risk evaluation techniques. A discussion of possible risk is presented in the form of a perspective on the possible magnitude of risk from environmental exposure. The perspective discussion notes the small exposure margins and possible overlap between some occupational and environmental exposure levels. This lessens the uncertainty of extrapolating the occupational hazard and observed risk into the environmental setting. Furthermore, based on a more quantitative approach involving the observed lung cancer from occupational exposures and the magnitude of occupational and environmental exposure differences, an exploratory risk analysis shows that environmental cancer risks possibly range from 10^{-5} to nearly 10^{-3}, while a

consideration of the numerous uncertainties and assumptions also indicates that lower risk is possible and zero risk cannot be ruled out. These risk findings are only general indicators of the potential significance of the lung cancer hazard and should not be viewed as a definitive quantitative characterization of risk or be used to estimate an exposure-specific population impact, i.e., estimating numbers of cancer deaths. Best scientific judgment guided the selection of assumptions and other elements of the analysis which are deemed reasonable and appropriate for identifying possible risks based on the information currently available. Further research is needed to more accurately assess and characterize environmental cancer risks of DE.

This exploratory risk analysis uses data collected from engines built prior to the mid-1990s. While engine exhaust emissions have been decreasing and exhaust composition has been changing in recent years, particularly for on-road engines, EPA believes that the insight gained from the risk perspective is pertinent to current on-road and nonroad engine use. New and cleaner engines will become available in response to environmental concerns and strict new regulations. As cleaner engines replace a substantial number of existing engines, the risk perspective will need to be reevaluated.

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9. CHARACTERIZATION OF POTENTIAL HUMAN HEALTH EFFECTS OF DIESEL EXHAUST: HAZARD AND DOSE-RESPONSE ASSESSMENTS

9.1. INTRODUCTION

Human health risk assessment entails the evaluation of all pertinent information on the hazardous nature of environmental agents, on the extent of human exposure to them, and on the characterization of the potential risk to an exposed population. The information is typically organized into four components: hazard assessment, dose-response assessment, exposure assessment, and risk characterization. This health assessment document focuses only on the hazard and dose-response assessment components. The overall objectives of this diesel engine exhaust (DE) assessment are:

- to identify and characterize the human health effects, i.e., hazards that may result from environmental exposure to DE;
- to determine whether there is a quantitative exposure/dose-response relationship for DE exposure and the health effect in the range of observation, and if sufficient data are available (1) for noncancer effects to derive estimates of exposure that are believed to be without appreciable risk, and (2) for carcinogenicity to derive an exposure/dose-specific cancer unit risk; and
- to summarize and integrate the findings of the assessment into a characterization and discuss the uncertainties.

This chapter summarizes and integrates the key findings about the nature and characteristics of environmental exposure to DE (Chapter 2), health hazard information (Chapters 3, 4, 5, and 7), and dose-response analyses (Chapters 6 and 8) that are relevant to the potential human health effects associated with current-day environmental exposure to DE. It also discusses the uncertainties associated with the key findings, including critical data and knowledge gaps, key assumptions, and EPA's science policy choices that are used to bridge the data and knowledge gaps.

This assessment is the Agency's first health assessment for DE emissions and was developed to provide information about the potential for DE-related environmental health hazards that could be used in evaluating regulatory initiatives under provisions of the Clean Air Act.

9.2. PHYSICAL AND CHEMICAL COMPOSITION OF DIESEL EXHAUST

As reviewed in Chapter 2, DE is a complex mixture of hundreds of constituents in gas or particle phases. Gaseous components of DE include carbon dioxide, oxygen, nitrogen, water vapor, carbon monoxide, nitrogen compounds, sulfur compounds, and low molecular-weight hydrocarbons and their derivatives. The particulate matter of DE, diesel particulate matter (DPM), is composed of elemental carbon (EC), adsorbed organic compounds, and small amounts of sulfate, nitrate, metals, trace elements, water, and unidentified compounds. Incomplete combustion of fuel hydrocarbons as well as engine oil and other fuel components such as sulfur leads to the formation of DPM. DPM is either directly emitted from diesel-powered engines (primary particulate matter) or is formed from the gaseous compounds emitted by a diesel engine (secondary particulate matter).

DE emissions vary in chemical composition and particle sizes among different engine types, fuel formulations, and within engine types according to operating conditions. As the emissions age in the environment they also change. There also have been changes in DE emissions over time as a result of changes in engine technology and fuel reformulation. The following sections identify and characterize the key components of DE that are of concern in possible health outcomes, and discuss the changes in the composition of DE over time. The latter information is critical for making a scientific judgment about the appropriateness of using epidemiologic and toxicologic findings from past DE exposures to assess hazard and risk from current-day environmental exposures. It should be noted that available animal studies are based on exhaust exposures from various model year on-road diesel engines since 1980, whereas many of the epidemiologic studies refer to exposures from on-road and nonroad diesel engines in use from the 1950s through the mid-1990s.

After emission from the tailpipe, DE undergoes dilution, chemical and physical transformations, and dispersion and transport into the atmosphere. After a day or so in the ambient environment, the exhaust mixture is said to be aging, a recognition of the atmospheric transformation processes, mostly focused on the organics present, though some particle size changes also may occur. The public health impact of DE mixture transformations is not clear, as some atmospheric processes alter chemical forms to a less toxic form whereas others seem to produce a chemical form with increased toxicity (Chapter 2, Section 2.3).

9.2.1. Diesel Exhaust Components of Possible Health Concern

The components of DE that are of health concern for this assessment are the particles (elemental carbon core), the organic compounds adsorbed to the particles, and the organic compounds present in the gas phase. The gaseous oxides of carbon, nitrogen, and sulfur are also

of public health interest and the relevant health considerations for these are reviewed separately in EPA's Ambient Air Quality Criteria Documents.

9.2.1.1. *Diesel Particles*

Approximately 80%-95% of DPM mass is in the fine particle size range (≤2.5 micrometers, ambient particulate matter [PM]), with a mean particle aerodynamic diameter of about 0.2 micrometers. Ultrafine particles (<0.1 micrometers), a smaller size component of the fine particles, average about 0.02 micrometers in aerodynamic diameter and account for about 1%-20% of the DPM mass and 50%-90% of the total number of particles in DPM (Chapter 2, Section 2.2.8.3).

Particle size is important for a number of reasons. Particles with aerodynamic diameters >2.5 micrometers (i.e., >$PM_{2.5}$) tend to be retained in the upper portions of the respiratory tract, whereas particles with diameters <2.5 micrometers (i.e., $PM_{2.5}$) are deposited in all areas, especially into the lower portions of the respiratory tract, including the deep lung. These fine and ultrafine particles have a very large surface area per gram mass (Chapter 2, Section 2.2.2), which enables them to adsorb and transport inorganic and organic compounds into the lung (Chapter 3, Section 3.3).

DPM is part of ambient particulate matter (PM). The EPA Emissions Trends Report (U.S. EPA, 2000) indicates that annual nationwide emissions of diesel $PM_{2.5}$ (on-road and nonroad) in 1998 were 77% of all mobile-source emissions in 1998, 23% of the total $PM_{2.5}$ inventory excluding natural and miscellaneous sources, and 6% if the natural and miscellaneous sources are included. Some geographic areas have a higher percentage of DPM in ambient $PM_{2.5}$ because of differences in the number and types of diesel engines present in the area (e.g., on-road engines as well as nonroad engines). For instance, in Manhattan, New York, on-road diesel PM was reported to contribute about 53% of ambient PM_{10} during 3 days in 1993, whereas 1996-1997 studies in the Phoenix and Denver areas showed diesel PM to be 10%-15% of total $PM_{2.5}$ mass (Chapter 2, Section 2.4.2.1).

DPM generally contains a high percentage of EC per unit mass, which can be used as a distinguishing feature from noncombustion sources of $PM_{2.5}$ and, to an extent, other combustion sources. The DPM EC content can range from more than 50% to approximately 75% of the DPM mass depending on age of engine, type of engine (heavy-duty versus light-duty), fuel characteristics, and driving conditions. The organic carbon portion of DPM can range approximately from 19% to 43%, though higher and lower values also have been reported. In comparison, gasoline engine exhaust generally has a reverse pattern of low EC content and a high percentage of organics on the particle mass (see Chapter 2, Table 2-13).

9.2.1.2. *Organic Compounds*

The organic compounds present in the gases and adsorbed onto the particles include a wide spectrum of compounds related to unburned diesel fuel, lube oil, low levels of partial combustion, and pyrolysis products (see Chapter 2, Table 2-19). The organic compounds present in the gaseous phase include alkanes, alkenes, aldehydes, monocyclic aromatic compounds, and polycyclic aromatic hydrocarbons (PAHs). Among the gaseous components of DE, the aldehydes are particularly important because of their potential carcinogenic effects and because they make up an important fraction of the gaseous emissions. Formaldehyde accounts for a majority of the aldehyde emissions (65%-80%) from diesel engines. Acetaldehyde and acrolein are the next most abundant aldehydes. Other gaseous components of DE that are notable for their carcinogenic effects include benzene, 1,3-butadiene, PAHs, and nitro-PAHs (including those with ≤4 rings and nitro-PAHs with 2 and 3 rings). A number of the gaseous compounds (e.g., aldehydes, alkanes, alkenes, NO_x, SO_x) also are known to induce respiratory tract irritation given sufficient exposure (see Chapter 2, Table 2-21). Very small amounts of dioxins have been measured in heavy-duty diesel truck exhaust. These emissions are estimated to represent about 1.2% of the 1995 national dioxin inventory; dioxin emissions from nonroad exhausts have not been estimated (Chapter 2, Section 2.2.7.2).

Organic substances adsorbed onto DPM include C_{14-35} hydrocarbon compounds, PAHs with ≥4 rings, and nitro-PAHs. PAHs and their derivatives comprise <1% of the DPM mass (Chapter 2, Section 2.2.8). Many of these hydrocarbons are known to have mutagenic and carcinogenic properties. California EPA (Cal EPA, 1998) identified at least 19 hydrocarbons present in DE that are known or suspected carcinogens, according to evaluations by the International Agency for Research on Cancer (IARC).

9.2.2. "Fresh" Versus "Aged" Diesel Exhaust

Newly emitted exhaust is termed "fresh," whereas exhaust that is more than 1 or 2 days old is referred to as "aged" because of alterations caused by sunlight and other chemical physical reactions that occur in the atmosphere. The overall toxicological consequence of DE aging is unclear because during aging some compounds in the DE mixture 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 toxic nitro-PAHs present in fresh exhaust. On the other hand, PAHs present in the gas phase can react with hydroxyl radicals present in the ambient air, leading to a reduced atmospheric lifetime of the original PAHs. Alkanes and alkenes may be converted to aldehydes, and oxides of nitrogen to nitric acid (Chapter 2, Section 2.3).

9.2.3. Changes in Diesel Exhaust Emissions and Composition Over Time

Chapter 2, with its Summary in Section 2.5, provides a full review of emissions trends and a complete characterization of the physical and chemical changes in DE over the years, taking into consideration the lack of consistent analytical and measurement techniques and the variability in emissions based on vehicle mix, driving cycles, engine deterioration, and other factors. Key findings and inferences relevant to the potential health effects of DE are discussed below.

As discussed in Chapter 2, Section 2.2.3, the EPA Emissions Trend Report estimates that DPM_{10} on-road emissions decreased 27% between 1980 and 1998. DPM emission factors (g/mile by model year) from new on-road diesel vehicles decreased on average by a factor of six from the mid-1970s to the mid-1990s. These significant reductions are largely attributable to reductions in three PM components: EC, organic carbon, and sulfate. Limited data are available to assess the changes in emission rates from locomotive, marine, or other nonroad diesel sources over time, although it is estimated that DPM_{10} (≤ 10 μm) emissions from nonroad diesel engines increased 17% between 1980 and 1998 (Chapter 2, Section 2.2.5).

Because of changes in engine technology and fuel composition, the chemical composition of DPM from on-road vehicles has also changed over time. The percentage of soluble organic material associated with DPM decreased by model year from the 1980s to the 1990s, and the proportion of EC is correspondingly higher. PAHs and nitro-PAHs are present in DPM from both new and older diesel engines. There are insufficient data to provide clear insight into the potential for changes in total PAH emissions over time or specific PAHs such as benzo[a]pyrene and 1-nitropyrene. It should be noted that the chemical composition of ambient DPM to which people are currently exposed is determined by a combination of exhaust from older and newer engines as well as on-road and nonroad applications of those engines. Consequently, the decrease in the soluble organic fraction of DPM by model year for on-road engines does not directly translate into a proportional decrease in DPM-associated organic material to which people are exposed. In addition, the contributions from high-emitting and/or smoking diesel engines have not been quantified (Chapter 2, Section 2.5.2).

Because of these uncertainties, the exposure impact of changes in DPM composition over time cannot be confidently characterized. Available data clearly indicate that toxicologically significant organic components of DE (e.g., PAHs, PAH derivatives, nitro-PAHs) were present in DPM and DE in the 1970s and are still present. Even though a significant fraction of ambient DPM (possibly more than 50%) is emitted by nonroad equipment, data are currently inadequate to characterize changes in the chemical composition of DPM from nonroad equipment over time. Given the variation in fuel, engine technology, and in-use operational factors over the years, caution should be exercised in presuming that a decrease in the amount of emissions or emission

constituents from older engines to present day in-use engines will result in a decrease in hazard/risk. In meeting the 2007 federal regulations for heavy-duty DE, the exhaust composition will be markedly changed with a consequence that health hazards are expected to be significantly reduced.

9.3. AMBIENT CONCENTRATIONS AND EXPOSURE TO DIESEL EXHAUST

Chapter 2, Section 2.4 provides information on occupational and environmental exposures to DE in order to provide a context for the hazard assessment and dose-response analysis. Highlights of the available information are discussed below.

DE is emitted from a variety of sources, both on-road (e.g., motor vehicles, construction equipment) and nonroad (e.g., farm equipment, railway locomotives, or marine uses). Environmental exposure to DE is generally higher in urban areas than in rural areas. The concentration of DE in the air will vary within any geographic area depending on the number and types of diesel engines in the area and the atmospheric patterns of dispersal. Some important factors that determine the difference between the ambient concentration of DE and the resultant exposure to an individual include the proximity of a person to the DE source and his/her pattern of activity which, for example, includes outdoor versus indoor activities as well as related breathing rates. Certain occupational populations (e.g., transportation and garage workers, heavy-equipment operators, and others who spend considerable time outdoors) can be exposed to much higher levels of DE than the general population. The amount or number of particles delivered and retained in the lung is one factor that could contribute to differential human susceptibility to DPM. For example, children have smaller lungs than adults and thus could have a higher lung burden of inhaled DPM per lung surface area if their activity pattern results in a high breathing frequency.

As DE is a complex mixture of many constituents, environmental concentration measurements and related human exposure is difficult to precisely measure. Even though levels of a number of DE constituents are generally known, it is difficult to quantify the portion that comes from DE since other types of emission sources also may emit the same constituent. Moreover, there is still incomplete knowledge about the relative roles of the relevant DE constituents in mediating the potential health effects of DE. Historically, exposure levels to DPM have been used as a surrogate marker/dosimeter for whole DE. Although uncertainty exists as to whether DPM mass (expressed as $\mu g/m^3$ of DPM) is the most appropriate dosimeter for health effect purposes, it is considered to be a reasonable choice until more definitive information is available about the mechanisms or mode(s) of toxicity action of DE.

Several techniques exist for estimating ambient concentrations of DPM, including chemical mass balance (CMB) source apportionment, dispersion modeling, and using EC as a

surrogate for DPM. DPM concentrations reported from CMB and dispersion modeling studies in the 1980s suggest that in urban and suburban areas (Phoenix and Southern California), the annual average DPM concentration ranged from 2 to 13 $\mu g/m^3$. In the 1990s, annual or seasonal average DPM concentrations in suburban or urban locations have ranged from 1.2 to 4.5 $\mu g/m^3$. DPM concentrations at a major bus stop in downtown Manhattan ranged from 13.2 to 46.7 $\mu g/m^3$ over a 3-day period in 1993. In nonurban and rural areas in the 1980s, DPM concentrations were reported to range from 1.4 to 5 $\mu g/m^3$. In the 1990s, nonurban air basins in California were reported to have DPM concentrations ranging from 0.2 to 2.6 $\mu g/m^3$ (Chapter 2, Section 2.4.2).

A comprehensive exposure assessment is not presented in this assessment, though EPA is developing this in an analysis called the National Air Toxics Assessment. Interim exposure estimation based on EPA's Hazardous Air Pollutant Exposure Model (HAPEM-MS3 model), for on-road sources only, suggests that in 1996 annual average DPM exposure in urban areas from only on-road engines was 0.7 $\mu g/m^3$, while in rural areas exposure was 0.3 $\mu g/m^3$. Among 10 urban areas, the 1996 annual average estimated exposure ranged from 0.5 to 1.2 $\mu g/m^3$. A high-end exposure estimate for 1996 is not yet available. Comparable 1990 exposure estimates for on-road sources ranged from 0.9 $\mu g/m^3$ for urban areas to 0.5 $\mu g/m^3$ for rural areas. In 1990 exposure estimates for the most highly exposed individuals (e.g., outdoor workers and children who spend large amounts of time outdoors) were estimated to be up to 4.0 $\mu g/m^3$ (Chapter 2, Section 2.4.3.2, Table 2-29). Nationwide level nonroad emission exposures are estimated to be nearly double those from on-road sources.

Estimates for occupational exposures to DE as DPM mass are generally higher than environmental exposures. Tables 2-27 and 2-28 provide historic exposure estimates for specific worker categories. For example, historic DPM exposure estimates range from 39–191 $\mu g/m^3$ for railroad workers, 4–748 $\mu g/m^3$ for firefighters, 7–98 $\mu g/m^3$ for public transit workers and airport crews, 5–61 $\mu g/m^3$ for mechanics and dock workers, and 2–7 $\mu g/m^3$ for long- and short-haul truck drivers. For a direct comparison of lifetime exposures between an occupational setting (8 hours per day, 5 days per week, for 45 years) and environmental exposure (continuous exposure for 70 years), the occupational estimates are converted to an equivalent environmental lifetime estimate,[1] which is also shown in Table 2-28. A conversion of EC-based measurements to total DPM also may be needed for some estimates. The estimated 70-year lifetime exposures equivalent to those for the occupational groups discussed above range from about 0.4–157 $\mu g/m^3$. These data indicate that some lower-end occupational estimates of DPM, when converted to environmental equivalents, overlap the range of estimated environmental exposures

[1]Environmental equivalent occupational exposure = 0.21 × occupational exposure.

to DPM from on-road emissions (national average in 1990 of 0.8 $\mu g/m^3$, with high-end exposures up to about 4 $\mu g/m^3$). The addition of nonroad emission exposures, when appropriate, makes the case for overlap of occupational and environmental exposure more prevalent.

9.4. HAZARD CHARACTERIZATION

The primary health effects of concern from environmental exposure to DE include effects associated with both acute and short-term exposures as well as chronic exposures. It is recognized that acute exposures may produce transitory physiological symptoms of varied severity as well as exacerbation of allergenic effects from acute and repeated exposures. On the basis of combined human and experimental evidence from chronic exposure studies, noncancer respiratory effects and lung cancer are observed.

The health effects data are based on DE from a variety of engines existing before the mid-1990s. There have been changes in the physical and chemical composition of some DE emissions (on-road vehicle emissions) over time, though there is no definitive information to show that the emission changes portend significant toxicological changes. The mode(s) of action for DE toxicity in humans is not understood, and hence knowledge is lacking about the role of exhaust mixture components in modulating the toxicity. Taken together, these considerations have lead to a judgment that the hazards identified from older technology-based exposures are applicable to current-day exposures. As new and cleaner diesel engines replace a substantial number of existing engines, the general applicability of the older data will need to be reevaluated.

As discussed in Chapter 6 (Section 6.4), it is also reasonable to expect that DPM, being a constituent of ambient fine PM ($PM_{2.5}$), would contribute to the wider spectrum of effects that have been associated with ambient $PM_{2.5}$. Community epidemiologic studies have shown that ambient $PM_{2.5}$ exposure is statistically associated with increased mortality (especially among people over 65 years of age with preexisting cardiopulmonary conditions) and morbidity as measured by increases in hospital admissions, respiratory symptom rates, decrements in lung function, and exacerbation of asthma, and possibly immunological effects in the respiratory system. There continues to be little epidemiologic evidence for an effect of ambient exposure to PM on cancer rates (U.S. EPA, 1996a,b), though U.S. EPA's Criteria Document for Ambient PM (expected to be released in 2002) will examine the question further.

9.4.1. Acute and Short-Term Exposures

The combined human and animal evidence indicates that DE can induce irritation to the eye, nose, and throat, as well as inflammatory responses in the airways and the lung following

acute and/or short-term exposure to high concentrations. There also is suggestive evidence for possible immunological and allergenic effects of DE.

9.4.1.1. *Acute Irritation*

DE contains various respiratory irritants in the gas phase and in the particulate phase (e.g., SO_x, NO_x, aldehydes). Acute exposure to DE has been associated with irritation of the eye, nose, and throat, respiratory symptoms (cough and phlegm), and neurophysiological symptoms such as headache, lightheadedness, nausea, vomiting, and numbness or tingling of the extremities. Such symptoms have been described mainly in reports of individuals exposed to DE in the workplace, or in clinical studies in humans exposed acutely to high concentrations of DE. Because of the general lack of validating exposure information in the reports, the role of DE in causing these effects is unknown. An exposure-response relationship for these acute irritation and respiratory symptoms has not been demonstrated (Chapter 5, Section 5.1.1.1).

9.4.1.2. *Respiratory Effects*

Available studies of occupational exposure to DE have not provided evidence for significant decrements of lung function in workers over a work shift or after a short-term exposure period. Short-term and subchronic inhalation studies of DE in animals (rats, mice, hamsters, cats, guinea pigs) showed inflammation of the airways and minimal or no lung function changes. These effects were associated with high DE exposures (up to 6 mg/m^3). Exposure-response relationships have not been established for these responses (Chapter 5, Sections 5.1.1, 5.1.2, and 5.1.3).

9.4.1.3. *Immunological Effects*

Recent human and animal studies show that acute DE exposure episodes can exacerbate immunological reactions to other allergens or initiate a DE-specific allergenic reaction. The effects seem to be associated with both the organic and carbon core fraction of DPM. In human subjects, intranasal administration of DPM has resulted in measurable increases of IgE antibody production and increased nasal mRNA for some proinflammatory cytokines. These types of responses also are markers typical of asthma, though for DE, evidence has not been produced in humans that DE exposure results in asthma. The ability of DPM to act as an adjuvant to other allergens also has been demonstrated in human subjects. For example, co-exposure to DPM and ragweed pollen was reported to significantly enhance the IgE antibody response and cytokine expression relative to ragweed pollen alone. Available animal studies also demonstrate the potential adjuvant effects of DPM with model allergens, e.g., in mouse studies the allergenic reaction to ovalbumin and Japanese cedar pollen (Chapter 5, Sections 5.1.1.1.3 and 5.1.1.1.4).

Additional research is needed to further characterize immunological effects of DE and to determine whether or not the immunological effects constitute a low-exposure hazard. This health endpoint is of considerable public health concern, given the increases in allergic hypersensitivity in the U.S. population (Chapter 5, Section 5.6.2.6).

9.4.2. Chronic Exposure

9.4.2.1. *Noncancer Effects*

Available long-term and cross-sectional human studies have provided evidence for an association between respiratory symptoms (cough and phlegm) and DE exposure, but there was no consistent effect on lung function. DE has been shown in many animal studies of several species to induce lung injury (chronic inflammation and histopathologic changes) following long-term inhalation exposure. DE also has been tested in laboratory animals for other health effects, but no significant effects have been found. Overall, available data lead to the conclusion of a potential chronic respiratory hazard to humans from long-term exposure to DE.

9.4.2.1.1. ***Respiratory effects.*** A few human studies in various diesel occupational settings suggest that DE 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 available human studies limit their usefulness in drawing any firm conclusions about DE exposure and noncancer respiratory effects (Chapter 5, Section 5.1.1.2).

Available studies in animals, however, provide a large body of evidence demonstrating that prolonged inhalation exposure to high concentrations of DE can result in pulmonary injury. A number of long-term laboratory studies in rats, mice, hamsters, cats, and monkeys found varying degrees of adverse lung pathology including focal thickening of the alveolar walls, replacement of Type I alveolar cells by type II cells, and fibrosis. The rat is the most sensitive animal species to DE-induced pulmonary toxicity (Chapter 5, Sections 5.1.3 and 5.4).

Available mechanistic data, mainly in rats, indicates that the DPM fraction of DE is a controlling factor in the etiology of pulmonary toxicity, although a role for the adsorbed organic compounds on the particles and in the gaseous phase cannot be ruled out. The lung injury appears to be mediated by an invasion of alveolar macrophages that release chemotactic factors that attract neutrophils and additional alveolar macrophages, which in turn release mediators (e.g., cytokines, growth factors) and oxygen radicals. These mediators result in persistent inflammation, cytotoxicity, impaired phagocytosis, clearance of particles, and eventually deposition of collagen by activated fibroblasts. This mode of action seems to be operative for a variety of poorly soluble particles in addition to DPM (ILSI, 2000). Because long-term exposure

to DE has been shown to induce exposure-dependent chronic respiratory effects in a wide range of animal species, and the mode of action is deemed relevant to humans, there is a sufficient scientific basis to support a conclusion that humans also could be at hazard for these effects under a chronic exposure condition.

9.4.2.1.2. ***Other noncancer effects.*** The negative results from available studies in several animal species (rats, mice, hamsters, rabbits, monkeys) indicate that DE is not likely to pose a reproductive or developmental hazard to humans. There has been some evidence from animal studies indicating possible neurological and behavioral effects, as well as liver effects. These effects, however, are seen at exposures higher than the respiratory effects. Overall, there is insufficient evidence to conclude that a low-exposure hazard exists for these endpoints (Chapter 5, Section 5.1.3.3).

9.4.2.2. *Carcinogenic Effects*

Many epidemiologic and toxicologic studies have been conducted to examine the potential for DE to cause or contribute to the development of cancer in humans and animals, respectively. In addition, there are some mode-of-action studies that seek to provide an improved understanding about the underlying carcinogenic process and thus contribute to a better understanding of the likelihood of hazard to humans. The available evidence indicates that chronic inhalation of DE is likely to pose a lung cancer hazard to humans. There is insufficient information for an evaluation of the potential cancer hazard of DE by oral and dermal routes of exposure.

9.4.2.2.1. ***Epidemiologic studies.*** Twenty-two epidemiologic studies about the carcinogenicity of workers exposed to DE in various occupations are reviewed in Chapter 7, Section 7.2. Exposure to DE has typically been inferred on the basis of job classification within an industry, with cumulative exposure based on duration of employment or age. Increased lung cancer risk, although not always statistically significant, has been observed in 8 out of 10 cohort studies and 10 of 12 case-control studies within several industries, including railroad workers, truck drivers, heavy-equipment operators, farm tractor operators, and professional diesel vehicle drivers. The increased lung cancer relative risks generally range from 1.2 to 1.5, although a few studies show relative risks as high as 2.6. Statistically significant increases in relative risk, 1.33 to 1.47, are also shown in two independent meta-analyses. The meta-analyses demonstrate the effect of pooling many studies and in this case show the positive relationship between DE exposure and lung cancer across a variety of DE-exposed occupations.

The generally small increases in lung cancer relative risk (1.2 to 1.5, i.e., less than 2) observed in the epidemiologic studies potentially weakens the evidence of causality. This is because with a relative risk of less than 2, if confounders (e.g., smoking, asbestos exposure) were having an effect on the observed risk increases, then it could be enough to account for the increased risk. With the strongest risk factor for lung cancer being smoking, there is a lingering uncertainty as to whether smoking effects may be influencing the magnitude of the observed increased relative risks, in spite of the fact that in key studies the investigating epidemiologists assert that they have effectively controlled for smoking. In studies in which the effects of smoking were controlled, increased relative risks for lung cancer prevailed. While some studies did not have information on smoking, confounding by smoking is judged unlikely to be significant if the comparison populations were from the same socioeconomic class.

As evaluated in Chapter 7 (Section 7.2.4.5), application of the criteria for causality provides a rational basis to conclude that the increased risks observed in available epidemiologic studies are consistent with a causal association between exposure to DE and occurrence of lung cancer. Overall, the human evidence for potential carcinogenicity for DE is judged to be strong but less than sufficient to satisfy the criteria for a "known" human carcinogen because of exposure uncertainties (lack of historical exposure of workers to DE) and residual uncertainty as to whether all confounders have been satisfactorily accounted for. The epidemiologic evidence is inconclusive for DE being associated with other forms of cancer.

9.4.2.2.2. ***Animal studies.*** DE and its organic constituents, both in the gaseous and particle phase, have been extensively tested for carcinogenicity in many experimental studies using several animal species and with different modes of administration. Several well-conducted studies have consistently demonstrated that chronic inhalation exposure to sufficiently high concentrations of DE produced dose-related increases in lung tumors (benign and malignant) in rats. In contrast, chronic inhalation studies of DE in mice showed equivocal results, whereas negative findings were consistently seen in hamsters. The gaseous phase of DE (filtered exhaust without particulate fraction), was found not to be carcinogenic in rats, mice, or hamsters. The available data indicate that among the traditional animal test species, the rat is the most sensitive species to DE. As reviewed in Chapter 7, Section 7.4, the lung cancer response in rats from high-concentration exposures to DE appears to be mediated by impairment of lung clearance mechanisms owing to particle overload, resulting in persistent chronic inflammation and subsequent pathologic and neoplastic changes (i.e., cancer) in the rat lung. Particle overload conditions in the human lung are not expected to occur as a result of environmental or most occupational exposures to DE. Thus, the increased lung tumors in the rat are not an appropriate

basis from which to judge the potential for a human hazard or perform a dose-response analysis to derive a cancer unit risk for humans.

In several intratracheal instillation studies, DPM, DPM organic extracts, and carbon black, which is virtually devoid of PAHs, have been found to produce increased lung tumors in rats. When directly implanted into the rat lung, DPM condensate containing mainly four- to seven-ring PAHs induced increases in lung tumors. DPM extracts also have been shown to cause skin tumors in several dermal studies in mice and sarcomas in mice following subcutaneous injection. Overall there are consistent findings of carcinogenic activity by the organic extracts of DPM in noninhalation studies (i.e., intratracheal instillation, lung implantation, skin painting). This contributes to the evidence for a potential human hazard.

9.4.2.2.3. ***Other key data.*** While not as extensive as the human and animal carcinogenicity data, other types of data are judged to be supportive of DE's potential carcinogenicity in humans. As mentioned previously, DE is a complex mixture of hundreds of constituents in either the gaseous phase or particle phase. Although present in small amounts, several organic compounds in the gaseous phase (e.g., PAHs, formaldehyde, acetaldehyde, benzene, 1,3-butadiene) are known to exhibit mutagenic and/or carcinogenic activities. PAHs and PAH derivatives, including nitro-PAHs present on the diesel particle, also are known to be mutagenic and carcinogenic. As reviewed in Chapter 4, DPM and DPM organic extracts have been shown to induce gene mutations in a variety of high-dose bacteria and mammalian cell test systems. DPM and DPM organic extracts also have been shown to induce chromosomal aberrations, aneuploidy, and sister chromatid exchange in both rodent and human in vitro tests.

There also is suggestive evidence for the bioavailability of organic compounds from the DE mixture. Elevated levels of DNA adducts in lymphocytes have been reported in workers exposed to DE. In addition, inhalation studies of animals using radio-labeled materials indicate some elution of organic compounds from DPM after deposition in the lung as measured by their presence in biological tissue and fluids (Chapter 3, Section 3.5).

9.4.2.2.4. ***Modes of carcinogenic action.*** The term "mode of action" refers to a series of key biological events and processes that are critical to the development of cancer. As discussed in Section 9.4.2.2.2, there is an understanding of the modes of action for the DE-induced lung tumors in the rat. However, the modes of action by which DE increases lung cancer risks in humans are unknown, and the evidence in rats is not applicable to environmentally exposed humans.

As discussed in Chapter 7, Section 7.4, it is hypothesized that multiple modes of action could be involved in mediating the carcinogenic effect of DE. These modes of action may

include: (a) mutagenic events (e.g., direct effects on DNA and effects on chromosomes) by organic compounds in the gas and particle phase, (b) indirect DNA damage via the production of reactive oxygen species (ROS) induced by particle-associated organics, and (c) particle-induced chronic inflammatory response leading to oxidative DNA damage through the release of cytokines, ROS, etc., and an increase in cell proliferation.

In rats, the particulate phase appears to have the greatest contribution to the carcinogenic effects, and both the particle core and the associated organic compounds have demonstrated carcinogenic properties in one or more test systems. While limited rat data and comparative potency calculations suggest that gas-phase components are not the primary factors in the development of lung cancer, a contributory role of the recognized toxic components cannot be dismissed. The relative importance of the various modes of action may be different at different exposure levels. Evidence from rat studies indicates the importance of the EC component of the DE particle in mediating lung tumor response at high exposure levels. As for the particle-absorbed organics, their inherent toxicity potential gives rise to a hypothesis that they may play a role in low or high exposures to DE.

9.4.2.2.5. ***Weight-of-evidence evaluation.*** Chapter 7, Section 7.5, provides an evaluation of the overall weight of evidence for human carcinogenicity in accordance with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986, 1996a, 1999). The totality of evidence supports the conclusion that DE is a *probable human carcinogen (Group B1)* by inhalation exposure using the criteria in the 1986 guidelines. A cancer hazard narrative for DE also is provided in accordance with the revised draft 1996/1999 guidelines, which concludes that DE *is likely to be carcinogenic to humans* by inhalation from environmental exposures. The common bases for either conclusion include the following lines of evidence:

- strong but less than sufficient evidence for a causal association between DE exposure and increased lung cancer risk among workers in varied occupations where exposure to DE occurs;
- extensive supporting data including the demonstrated mutagenic and/or chromosomal effects of DE and its organic constituents, and knowledge of the known mutagenic and/or carcinogenic activity of a number of individual organic compounds present with particles and in the DE gases;
- evidence of carcinogenicity of DPM and the associated organic compounds in rats and mice by noninhalation routes of exposure; and
- suggestive evidence for the bioavailability of DE organics from DE in humans and animals.

A notable uncertainty in the characterization of the potential cancer hazard of DE at low levels of environmental exposure is the incomplete understanding of about its mode(s) of action for the induction of lung cancer in humans. Available data suggest that DE-induced lung carcinogenicity may be mediated by mutagenic and nonmutagenic events by both the particles and the associated organic compounds, and that a role for the organics in the gaseous phase cannot be ruled out. Given that there is some evidence for a mutagenic mode of action, a cancer hazard is presumed possible at environmental levels of exposure. This is consistent with EPA's science policy position that assumes a nonthreshold effect for carcinogens with a mutagenic component in the absence of definitive data demonstrating a threshold or nonlinear mechanism. Additional support for an environmental hazard also comes from a comparison of the estimated environmental levels to the estimated occupational exposure levels where risk is seen. Given that there is only a minimal margin between environmental and occupational exposure ranges, if not an overlap, the extrapolation of observable hazard from the occupational setting to the ambient environment is relatively confident. Because of insufficient information, the human carcinogenic potential of DE by oral and dermal exposures cannot be determined.

Several organizations previously have reviewed available relevant data and evaluated the potential human carcinogenicity of DE or the particulate component (DPM) of DE. Similar conclusions were reached by various organizations (see Table 7-9). For example, some organizations have concluded that DE is probably carcinogenic to humans (IARC, 1989; IPCS, 1996), or reasonably is anticipated to be a carcinogen (NTP, 2000).

Overall, the weight of evidence for potential human carcinogenicity for DE is considered strong, even though inferences are involved. Uncertainties are present, however, and include the following unresolved issues.

First, there has been a considerable scientific debate about the significance of the available human evidence for a causal association between occupational exposure and increased lung cancer risk. Some experts view the evidence as weak and/or inconsistent while others consider the evidence compelling. This is due to a lack of consensus about whether the effects of smoking and other potential confounders have been adequately accounted for in key studies, and the lack of agreed-upon historical DE exposure data for the available studies.

Second, while the mode of action for DE-induced lung tumors in rats from high exposure is sufficiently understood, the mode of action for the DE lung cancer risk in humans is not known. To date, available evidence for the role of both the adsorbed organics and the carbon core particle has only been shown under high-exposure experimental animal test conditions. There is virtually no information about the relative role of DE constituents in mediating carcinogenic effects at the low-exposure levels.

Additional research is needed to address these issues to reduce the uncertainty associated with the potential cancer hazard of exposure to DE.

The relevance of this hazard characterization to current ambient DE exposures hinges on recognizing that the health effects data are derived from engine technologies and fuels that existed in the past, and that some changes in the DE exhaust mixture have occurred and can be expected in the future. Although decreases in amount and changes in composition of DE emissions have occurred over time for on-road engines, a change is slow to manifest in the environment because, for example, vehicular fleet turnover is slow and the change is slow to dominate across an engine fleet. Available studies have not focused on the potential toxicological effect of the emission changes. There is no compelling evidence at present to show that past and present exhaust characteristics are so toxicologically dissimilar as to render the current use of the assessment's findings outdated.

9.5. DOSE-RESPONSE ASSESSMENT

In assessments of estimated human health risks, human data from environmental exposures are always preferred over animal data, if available, as their use obviates the need for extrapolation across species, e.g., from animals to humans. However, for most environmental agents, available health effects information is generally limited to occupational exposures in studies of humans (e.g., workers) or high experimental exposures to laboratory animals. For the agents with high-exposure data compared to environmental exposure levels of interest, dose-response assessment is performed in two steps: assessment of data in the observable range to derive a point of departure (which usually is the lowest exposure or dose that induces some, minimal, or no apparent effects), followed by extrapolation to lower exposures to the extent necessary. Extrapolation to low exposures is ideally based on the understanding of mode(s) of toxic action of the agent which allows the development and use of a mode of action specific exposure-response model. In the absence of sufficient data, default methods and models are used to extrapolate to the lower exposure levels.

For DE, there is sufficient evidence to conclude that acute or short-term inhalation exposure at relatively high levels can cause irritant effects to the eye, nose, and throat, respiratory symptoms, and neurophysiological symptoms such as headache, nausea, etc., however, no quantitative data are available to derive an estimate of human exposure that is not likely to elicit irritant and inflammatory effects in humans.

There is also sufficient evidence to support the conclusion that DE has the potential to cause cancer and noncancer effects of the lung from long-term inhalation exposure. Chapters 6 and 8 provide dose-response analyses related to the noncancer and cancer hazards to humans, respectively, from lifetime exposure to DE. A dose-response analysis to estimate the expected

response at environmental exposure levels has less uncertainty the closer the animal test or estimated human epidemiologic-related exposures are to the environmental levels of interest. With increasing exposure margins (EM), and thus a greater range of extrapolation, the uncertainty about the shape of the dose-response curve in the region of low-dose extrapolation increases and the possibility of a zero risk cannot be ruled out.

9.5.1. Evaluation of Risk for Noncancer Health Effects

As discussed previously (Section 9.4.2.1), the evidence for potential chronic noncancer health effects of DE is based primarily on findings from chronic animal inhalation studies showing a spectrum of dose-dependent chronic inflammation and histopathological changes in the lung in several animal species including rats, mice, hamsters, and monkeys. A limited number of epidemiologic investigations of workers exposed to DE have not provided consistently clear evidence of significant chronic respiratory effects associated with DE exposure. On the other hand, the relatively large epidemiologic database for ambient PM shows a clear relationship between respiratory effects and ambient fine PM that is partially composed of DPM. The specific role of DPM or any other source-related constituent of ambient PM in causing the observed respiratory effects has not been defined.

The approach taken in this assessment to estimate a level of DE in the air to which humans may be exposed throughout their lifetime without an appreciable risk of deleterious effects is to derive a reference concentration (RfC) for DE based on the consistent data for respiratory inflammation in the rat studies. This approach assumes that humans would respond to DE similarly to the tested animals under similar exposure conditions. An uncertainty of this approach stems from the circumstance that animal studies have used high DE exposures, and the animal results must be translated to humans as well as to lower exposure levels since the potential chronic health effects of DE in humans at environmental exposure levels cannot be ascertained from the available DE human data.

It also is relevant to recognize that DPM is a component of ambient fine PM and that there is a relative wealth of human effects data for ambient PM showing a similarity of certain adverse health effects for DPM and ambient fine PM. This allows one to reasonably expect that the $PM_{2.5}$ National Ambient Air Quality Standard (NAAQS) would provide a measure of protection from DPM, reflecting DPM's current and approximate proportion to $PM_{2.5}$. Ambient $PM_{2.5}$ has been shown to be statistically associated with increased mortality (especially among people over 65 years of age with preexisting cardiopulmonary conditions) and morbidity, as measured by increases in hospital admissions, respiratory symptom rates, and decrements in lung function with both long- and short-term changes in ambient $PM_{2.5}$ concentrations.

9.5.1.1. *Chronic Reference Concentrations for Diesel Exhaust*

An inhalation Reference Concentration (RfC) is based upon long-term data, i.e., chronic exposure, and can be derived from either human or animal data. An RfC is correctly 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 methodology assumes that there is an exposure threshold below which effects will not occur. The RfC is not a bright line; rather, as the long-term human exposure increases above the RfC, the margin of protection decreases.

With the absence of DE exposure-response data in humans, this assessment derives an RfC for DE based on dose-response data from four chronic inhalation studies in rats (Mauderly et al., 1987; Ishinishi et al., 1988; Heinrich et al., 1995; Nikula et al., 1995). All of these studies used DPM (expressed as $\mu g/m^3$) as a measure of DE exposure. The pulmonary effects, including inflamation and histopathologic lesions, were considered to be the critical noncancer effects. As shown in Table 6-2, the no-observable-adverse-effects levels (NOAELs), the lowest-observable-adverse-effects levels (LOAELs), and the adverse effects levels (AELs) for lung inflammation and histopathologic changes were identified for the first three studies. For the Nikula et al. study, lower 95% confidence estimates of the concentrations of DPM associated with a 10% incidence ($BMCL_{10}$) of chronic pulmonary inflammation and fibrosis were derived since NOAEL's were not observed. For all four studies, human equivalent concentrations (HECs) corresponding to the animal NOAEL, LOAEL, AEL, and $BMCL_{10}$ were then computed using a dosimetry model developed by Yu et al. (1991) as described in Chapter 6, Section 6.5.2, and Appendix A. The dosimetry model accounts for species differences (rat to human) 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 purpose is to identify the highest HEC value with no apparent effect, i.e., $NOAEL_{HEC}$.

The highest $NOAEL_{HEC}$ associated with no apparent effect is 144 $\mu g/m^3$ from the Ishinishi et al. (1988) study; this then becomes the point of departure for deriving an RfC. To obtain the RfC, this point of departure was divided by two types of uncertainty factors (UF): a factor of 3 recognizes interspecies (i.e., rat to human) extrapolation uncertainties, and a factor of 10 reflects uncertainties about interindividual human variation in sensitivity. An evaluation of the interspecies extrapolation issues for dosimeteric and pharmacodynamic equivalence between rats and humans showed that although some adjustments could be accounted for, there remained a residual uncertainty, and thus an uncertainty of 3 out of a possible factor of 10 is used. In the absence of mechanistic or specific data, a default value of 10 is considered appropriate to account for possible human variability in sensitivity, particularly for children and people with

preexisting respiratory conditions. The spectrum of the population that may have a greater susceptibility cannot be better characterized until there is additional knowledge about mode of action. The resulting RfC for DE is 5 $\mu g/m^3$ of DPM.

Overall, the confidence level in the RfC is considered medium in a range of low to high confidence. A principal uncertainty of the RfC analysis is the reliance on animal data to predict human risk. The critical effects, chronic inflammation, and pathologic changes, which are well characterized in four animal species, are considered relevant to humans. Collective evidence for all poorly soluble particles, including DPM, indicates that the rat is the most sensitive laboratory animal species tested to date. Although in general the rat is thought to be more sensitive to lung injury than humans to poorly soluble particles (ILSI, 2000), it is not clear that this is the case specifically for diesel. We must recall that DE is a mixture of not just carbon particles but also various organics, both on the particles and in gases. In addition, differences in particle deposition, retention, and clearance mechanisms have been largely but perhaps not completely addressed by the use of the rat-to-human dosimetry model. The use of rat data is not likely to grossly underestimate the human risk for pulmonary noncancer health effects. In terms of the potential for other critical health effects, there is growing evidence suggesting that DE can exacerbate allergenic effects to known sensitizers, while also evoking production of biochemical markers typically associated with asthma. Some work in this area indicates that humans may be as sensitive as rats and mice to the immunologic effects (Chapter 6, Section 6.3.4). This database is currently lacking key exposure-response data, but may in the future provide an alternative basis for RfC derivation. It also should be noted that the ambient PM health effects data show a broader array of adverse human health concerns (e.g., cardiovascular effects, as well as acute exposure effects). With DPM being a ubiquitous component of ambient PM, there is an uncertainty about the adequacy of the existing DE noncancer database to identify all of the pertinent DE-caused noncancer health hazards.

9.5.1.2. *Risks Based on Ambient $PM_{2.5}$*

As discussed in Chapter 6 (Section 6.4), in 1997 EPA established an annual NAAQS for $PM_{2.5}$, at a level of 15 $\mu g/m^3$ to provide protection against adverse health effects associated with both long- and short-term exposures to ambient fine PM. DPM is a typical constituent of ambient fine PM (generally about 10% of $PM_{2.5}$ with some examples up to 36%).[2] Given the

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[2]"A qualitative comparison of adverse effects of exposure to DPM and ambient fine PM shows that the respiratory system is adversely affected in both cases, though a wider spectrum of adverse effects has been identified for ambient fine PM. In contrast to the diesel PM database, there is a wealth of human data for fine PM noncancer effects which indicates that the health effects from fine PM do not have a discernable threshold at this time."

similarity of health concerns for respiratory inflammation and pulmonary health effects from both DPM and fine particles, it is reasonable to expect that DPM contributes to some of the health effects associated with $PM_{2.5}$. Current knowledge is insufficient, however, to describe the relative potencies of DPM and the other components of $PM_{2.5}$. As long as the percentage of DPM to total ambient $PM_{2.5}$ remains in similar proportion, protective levels for $PM_{2.5}$ would be expected to offer a measure of protection from effects associated with DPM.

9.5.1.3. *Conclusions*

This assessment estimates an exposure air level of DE (as measured by DPM) to which humans may be exposed throughout their lifetime without experiencing any adverse noncancer health effects. The approach taken applies the RfC method using data specific to DE to produce an RfC of 5 $\mu g/m^3$ of DPM on the basis of four chronic inhalation studies of DE in rats and a composite uncertainty factor of 30. In addition, this assessment also recognizes the relative wealth of data regarding health effects associated with ambient PM and presumes that a health protective level for $PM_{2.5}$ also would be expected to provide a measure of protection from DPM, a constituent part of $PM_{2.5}$. The $PM_{2.5}$ standard of 15 $\mu g/m^3$ as an annual average thus is expected to provide a measure of protection from DPM noncancer health effects, reflecting DPM's current approximate proportion to $PM_{2.5}$.

9.5.2. Evaluation of Cancer Risks

As discussed in Section 9.4.3, the combined weight of evidence indicates that DE has the potential to pose a cancer hazard to humans at anticipated levels of environmental exposure. The target organ of DE-induced carcinogenicity is the lung. Strong evidence exists for a causal relationship between risk for lung cancer and occupational exposure to DE in certain occupational workers such as railroad workers, truck drivers, heavy-equipment operators (e.g., shipyard, diesel farm equipment, and construction), and transit workers. The evidence, however, was less than sufficient to confidently characterize DE as carcinogenic to humans, and instead the assessment concludes that DE is likely to be a human carcinogen. It also has been shown unequivocally in several studies that DE can cause benign and malignant lung tumors in rats in a dose-related manner following chronic inhalation exposure to high concentrations; however, this response is not thought applicable to predict a hazard to humans exposed at lower environmental levels. The mechanism(s) by which DE would induce lung cancer in humans has not been established, but available data suggest that mutagenic and nonmutagenic modes of action are possible. Hence, for estimating DE cancer risk at low environmental exposures, linear low-dose extrapolation would be considered an appropriate default assumption, which is consistent with EPA's science policy position that in the absence of an understanding of modes of carcinogenic

action, a nonthreshold effect is to be presumed (U.S. EPA, 1986, 1996a). This same assumption has been used by other organizations/risk assessors who have previously used either linear risk extrapolation models or mechanistically based models to estimate cancer risk from environmental exposure to DE (e.g., WHO-IPCS, 1996; Cal EPA, 1998; also see Appendix C).

Dose-response assessment is based on either human or animal data, although human data are always preferred if available. Several quantitative assessments have been conducted by organizations and investigators on the basis of both occupational data and rat data (see Appendix C). However, more recent evidence indicates that DE causes tumors in the rat lung via a mode of action that involves impairment of lung clearance mechanisms (referred to as "lung overload response") associated with high exposures. This lung overload response is not expected in humans exposed to environmental levels (nor is it expected to occur in many occupational exposures), and thus the rat lung tumor dose-response data are not considered suitable for predicting human risk at low environmental exposures. Given that the rat data are not appropriate for estimating cancer risk to humans, this assessment focuses on using the occupational epidemiologic data for estimating environmental risk of DE to humans.

Even though occupational data are considered most relevant for use in dose-response assessment, uncertainties exist, including the following issues:

- the use of DPM (expressed as $\mu g/m^3$) as a surrogate dosimeter for DE exposure, given that the relative roles of various constituents in mediating carcinogenic effects and the mode of carcinogenic action are still unknown;
- the representativeness of occupational populations for the general population and vulnerable subgroups, including infants and children and individuals with preexisting diseases, particularly respiratory conditions;
- the lack of actual DE exposure data for workers in the available epidemiologic studies;
- possible confounders (smoking and asbestos exposure) that could contribute to the observed lung cancer risk in occupational studies of DE if the control for these confounders is not adequate; and
- whether or not an exposure-response relationship for occupational lung cancer risk can be estimated for DE.

Chapter 8, Section 8.3, provides a discussion of these uncertainties, along with an evaluation of the suitability of available occupational studies for a derivation of a cancer unit risk estimate for DE. Unit risk is defined as the estimated upper-bound cancer risk at a specific exposure or dose

from a continuous average lifetime exposure of 70 years (in this case, cancer risk per $\mu g/m^3$ of DPM).

Among the occupational studies, the railroad worker studies (Garshick et al., 1987, 1988) and the Teamsters Union truck driver studies (Steenland et al., 1990, 1998) are considered to have the best available exposure data for possible use in establishing exposure-response relationships and deriving a cancer unit risk. There have been different views on the suitability of these studies for estimating environmental cancer risks (e.g., Cal EPA, 1998; HEI, 1995, 1999). Given the equivocal evidence for the presence or absence of an exposure-response relationship for the study of railroad workers, and exposure uncertainties for the study of truck drivers, it is judged that available data are too uncertain at this time for the development of a confident quantitative dose-response analysis and subsequent derivation of cancer unit risk for DE.

In the absence of a cancer unit risk to assess population cancer risk, this assessment provides a "perspective" about the possible magnitude of risk in the population from environmental exposure to DE. One approach to estimating the possible magnitude of risk involves simply noting that risks to the general public would be low in comparison with occupational risk if the differences in the lower environmental exposures compared to the higher occupational exposures are large. If the differences are small, the environmental risks would approach the workers' risk observed in studies of past occupational exposures. A comparison of environmental equivalent occupational and ambient environmental exposures showed that for certain occupations, there is a potential for overlap between environmental exposure and the estimated environmental equivalent occupational exposure, while in other cases the environmental exposures could be up to about 100-fold lower than the occupational levels (see Table 8-1). For the exposure overlap case, one can infer that the environmental risk could be the same as, or approach, the risk magnitudes observed in the occupational studies. In the 100-fold lower case, the environmental risk could be about 100-fold lower than the observed risk magnitudes in the occupational studies. Risks to the general public are of potential concern when a significant risk is seen in the occupational setting and the difference between occupational and ambient exposure may overlap or is relatively small (within one to two orders of magnitude).

A second approach, which is related to the first approach but more quantitative, is to estimate possible ranges of lung cancer risk from occupational exposures to DE, and then use a proportional relationship of exposure differences (e.g., EMs) to scale the occupational risk to the environmental exposure setting. Given the range of observed relative risks or odds ratios of lung cancer in a number of occupational studies, a relative risk increase of 1.4 was selected as a reasonable estimate of occupational risk for the purpose of this analysis. The relative risk of 1.4

means that the workers faced an extra risk that is 40% higher than the approximate 5% background lifetime lung cancer risk in the U.S. population. Using the relationship [*excess risk = (relative risk-1) × background risk*], 2% of these DE-exposed workers (i.e., 10^{-2} risk) would have been at risk (and developed lung cancer) attributable to occupational exposure to DE.

Using a nationwide average environmental exposure (0.8 $\mu g/m^3$ DPM), and assuming (a) the excess lung cancer risk from occupational exposure is about 10^{-2}; and (b) the past occupational exposures were no higher than about 1,200 $\mu g/m^3$ (equivalent to an environmental equivalent EM of 315, connoting a relatively large EM), the environmental cancer risk would fall between 10^{-4} and 10^{-5}. The selection of 1,200 $\mu g/m^3$ is a very high value intentionally selected to illustrate a high-end exposure boundary and thus a lower bounding of risk calculated by this exploratory approach. On the other hand, if occupational exposures for some groups were lower, for example, closer to 100 $\mu g/m^3$ (equivalent to an environmental equivalent EM of 26, connoting a smaller EM), the environmental risk would be higher and approach 10^{-3}. The selection of 100 $\mu g/m^3$ is purposefully toward the lower end of the reported occupational exposure range which spans 7–403 $\mu g/m^3$ in Table 8-1. The risk estimates are attended by numerous uncertainties; their inclusion in this document does not constitute Agency endorsement of their validity as a surrogate for cancer unit risk; the range of values is not useful for estimating numbers of cancer cases; and the range of possible risk from environmental exposures also could be lower and a zero risk cannot be ruled out.

These types of exploratory analyses are not intended to be precise or provide a definitive characterization of cancer risk but are useful in illustrating and gauging the possible range of risk based on applying reasonable judgment. The analyses provides a sense of where an upper limit (or "upper bound") of the risk may be. These analyses are subject to uncertainties, particularly the lack of actual exposure information for the occupational epidemiologic studies and the use of public-health-conservative risk assessment assumptions. The possible risks also could be lower and a zero risk cannot be ruled out because (a) some individuals in the population may have a high tolerance to exposure from DE and therefore not be susceptible to cancer from environmental exposure, and (b) although not reported, there could be a threshold of exposure below which there is no cancer risk. Given these circumstances, we refer to this risk analysis as a "perspective" on possible risks. Best scientific judgment guided the selection of assumptions and other elements of this analysis which are deemed reasonable and appropriate for identifying possible risks based on the information currently available. Further research is needed to more accurately assess and characterize environmental cancer risks from DE.

9.6. SUMMARY AND CONCLUSIONS

The available health effects data show that acute (short-term episodic exposure) and chronic (long-term) exposure to DE can pose hazards to humans and that environmental exposures, in some cases, may have a risk.

At relatively high acute exposures, DE can cause acute irritation to the eye and upper respiratory airways and symptoms of respiratory irritation which may be temporarily debilitating. Evidence also shows that DE has immunological toxicity that can induce allergic responses (some of which are also typical of asthma) and/or exacerbate existing respiratory allergies. While the hazard potential is important for these acute and short exposure-related effects, quantitative dose-response estimates for these effects could not be developed because of the lack of exposure-response information.

It is concluded that long-term exposure to low levels of DE poses a hazard for chronic inflammation and pathological changes in the human lung. A level of human lifetime exposure thought to be without appreciable risk for lung damage is estimated to be 5 $\mu g/m^3$ of DPM, this being a calculated RfC value for DE. Because DPM is a constituent of ambient $PM_{2.5}$ and there is some similarity in potential adverse effects from DE and $PM_{2.5}$, it is expected that a measure of protection from health effects associated with DE is provided by the 1997 annual $PM_{2.5}$ NAAQS, set at a level of 15 $\mu g/m^3$.

DE is considered to pose a human lung carcinogenicity hazard, which is expressed in a weight-of-evidence conclusion that DE is judged to be a "probable" human carcinogen, or is "likely to be carcinogenic in humans by inhalation" at environmental or higher exposure conditions. Because of uncertainty in the available exposure-response data, a cancer unit risk/cancer potency for DE has not been derived. One should note that the closeness of the high-end environmental exposures and low-end estimates of occupational exposure suggest less uncertainty in the extrapolation of hazard and possible risk to the environmental setting. Exploratory analyses using public health conservative assumptions provides a perspective on the possible range of lung cancer risk from environmental exposure to DE. Best scientific judgment guided the selection of assumptions and other elements of this analysis which are deemed reasonable and appropriate for identifying possible risks based on the information currently available. These analyses indicate that lifetime cancer risk may exceed 10^{-5} and could be as high as 10^{-3} or nearly so, though considering the assumptions used and the uncertainties, lower risk is possible and a zero risk cannot be ruled out. This range of values is attended by numerous uncertainties, the inclusion of the range in this assessment does not constitute Agency endorsement of their validity as surrogates for cancer unit risk values, and the range is not suitable for estimating numbers of cancer cases. These risk findings should not be viewed as a definitive characterization of risk.

Even though the evidence for potential human health hazards for DE is convincing and persuasive, uncertainties exist because of the use of assumptions to bridge data and knowledge gaps about human exposures to DE and the underlying mechanisms by which DE may cause the observed toxicities in humans and animals. A notable uncertainty of this assessment is how the physical and chemical nature of DE emissions has changed over the years because the toxicological and epidemiologic observations are based on older engines and their emissions, yet the desire is to focus on the potential health hazards related to exposure from present-day or future emissions. There have been changes in the physical and chemical composition of some DE emissions (on-road vehicle emissions) over time, though there is no definitive information to show that the emission changes portend significant toxicological changes. The mode(s) of action for DE toxicity in humans is not understood, and hence knowledge is lacking about the role of exhaust mixture components in modulating the toxicity. Taken together, these considerations have lead to a judgment that the hazards identified from older technology-based exposures are applicable to current-day exposures. As new and cleaner diesel engines replace a substantial number of existing engines, the general applicability of the conclusions in this assessment will need to be reevaluated.

Other uncertainties include the assumptions that health effects observed at high doses may be applicable to low doses, and that toxicologic findings in laboratory animals are predictive of human responses. Also, the available data are not sufficient to demonstrate the absence or presence of an exposure/dose-response threshold in humans for DE toxicity at environmental exposures. Again, this is due in part to the lack of understanding of how DE may cause adverse health effects in exposed humans and laboratory animals. Although there are hypotheses about the specific mechanisms by which DE might cause cancer and other toxicities, no specific biological pathways or specific constituents of DE have been firmly established as responsible for low-dose effects. The assumptions used in this assessment, i.e., the presence of a biological threshold for chronic respiratory effects based on cumulative dosage and the absence of a threshold for lung cancer stemming from subtle and irreversible effects, are considered prudent and reasonable default choices.

The characterization of health hazards and risks contained in this document assumes that the potential DE health hazards are relevant for long-term exposures, up to and including lifetime exposures, and would apply to a wide spectrum of individuals but not necessary those that would have significant differential susceptibility. There is no DE-specific information that provides direct insight into the question of differential susceptibility within the general human population or vulnerable subgroups, for example, children or the elderly. Although default approaches to account for interindividual variation have been included in the derivation of the noncancer effects RfC (i.e., use of an uncertainty factor of 10), this may or may not adequately

protect certain subgroups that could be more vulnerable. Differential susceptibility to DPM among individuals in the population would be due to differences in dosimetry (i.e., differences in retained particle mass or number in the lung) and/or differences in respiratory system tissue response sensitivity. From the dosimetry perspective, we understand that age, gender, and disease status can influence deposition in the lung and other areas of the respiratory tract (U.S. EPA, 1996b, Section 10.7.7). For example, given that DE chronic toxicity is focused on the respiratory system, vulnerable subgroups might include those individuals who predispose their lungs to increased particle retention (e.g., smoking, high particulate burdens from nondiesel sources) or those having existing respiratory or lung inflammation, repeated respiratory infections, or chronic bronchitis or asthma. For children, there is also the hypothesis of possible increased sensitivity to exposure, given the ongoing processes of development from birth to maturation, of the respiratory and immune systems.

Despite the uncertainties regarding intraspecies variability, the default approach of using an uncertainty factor of 10 in the derivation of the noncancer effects RfC to account for possible interindividual variation in the toxic response to DE exposure is appropriate and reasonable given the lack of DE-specific data.

Variation in DE exposure is another source of uncertainty. Because of variation in human activity patterns and their proximity to DE sources of emissions, different population subgroups could potentially receive higher or lower exposure to DE. The highest exposed are clearly occupational subgroups whose job brings them very close to DE sources, such as diesel engine vehicle drivers and workers, diesel powered machinery operators, some underground miners, etc. High exposures in the general population would be to those living very near or having time outdoors in proximity to DE sources as well as those engaged in activities that cause high breathing rates where DE is present. Accordingly, where appropriate, analyses in this assessment have included possible high-end DE exposures in addition to the lower nationwide average exposure estimates.

Lastly, this assessment considers only potential heath effects from exposures to DE alone. DE exposure could be additive or synergistic to concurrent exposures to other air pollutants. For example, there is evidence that DPM that has been altered by being in the presence of ambient ozone significantly increases the rat lung inflammatory effect compared to DPM that was not subjected to ozone (Madden et al., 2000). This observation suggests a hypothesis that inflammation-related noncancer hazards of airborne DPM may be worsened by the increasing presence of ozone in the ambient air. Other concerns include the possible impacts for children and adults on the exacerbation of existing allergens resulting from DE exposure. However, in the absence of more definitive data demonstrating interactive effects

from combined exposures to DE and other pollutants, it is not possible to further address these issues at this time.

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Appendix A

Calculation of Human Equivalent Continuous Exposure Concentrations (HECs)

A.1. INTRODUCTION

As discussed in Chapter 3, the lung burden of diesel particulate matter (DPM) 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 appendix presents a mathematical model that simulates the deposition and clearance of DPM in the lungs of rats and humans of Yu et al.(1991) also published as Yu and Yoon (1990).

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 typically 0.2 μm and is polydisperse with a geometric standard deviation of around 2.3. The organics adsorbed onto the aggregates normally account for 10% to 30% of the particle mass. However, the exact size distribution of DPM 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 DPM have been reviewed extensively by Amann and Siegla (1982) and Schuetzle (1983).

Four mechanisms deposit DPM 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 and the other three mechanisms play minor roles.

Once DPM is deposited in the respiratory tract, both the carbonaceous core and the adsorbed organics will be removed from the deposition sites by mechanical clearance, provided by mucociliary transport in the ciliated conducting airways as well as macrophage phagocytosis and migration in the nonciliated airways, and dissolution. As the carbonaceous core or soot of DPM is insoluble, it is removed from the lung primarily by mechanical clearance, whereas the adsorbed organics are removed principally by dissolution (Chapter 3).

A.2. PARTICLE MODEL

To develop a mathematical model that simulates the deposition and clearance of DPM in the lung, an appropriate model for diesel particles must be introduced. For the deposition study, an equivalent sphere model developed by Yu and Xu (1987) was used to simulate the dynamics and deposition of DPM in the respiratory tract by various mechanisms. For the clearance study, a diesel particle is assumed to be 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 3. 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 general and made to account for the biphasic clearance of DPM; it does not specifically imply the actual nature of the adsorbed organics. For aerosols made of pure organics, such as benzo(*a*)pyrene (BaP) and nitropyrene (NP) in the same size range of DPM, 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 intracellularly (c) or covalently bound (d).

A.3. COMPARTMENTAL LUNG MODEL

The model of Yu et al. (1991) comprises three principal compartments involved in deposition and clearance: tracheobronchial (T or TB), alveolar (A), and lung-associated lymph node (L), as shown in Figure A-1. The outside compartments blood (B) and GI tract (G) and nasopharyngeal or head (H) are also represented. The alveolar compartment in the model is obviously the most 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 A-1, $r_H^{(i)}$, $r_T^{(i)}$ and $r_A^{(i)}$ are, respectively, the mass deposition rates of DE 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 f_i. Then

$$r_H^{(i)} = f_i \, r_H \, , \tag{A-1}$$

$$r_T^{(i)} = f_i \, r_T \, , \tag{A-2}$$

$$r_A^{(i)} = f_i \, r_A \, , \tag{A-3}$$

where r_H, r_T, and r_A are, respectively, the total mass deposition rates of DPM in the H, T, and A compartments, determined from the equations:

$$r_H = c(TV)(RF)(DF)_H \, , \tag{A-4}$$

$$r_T = c(TV)(RF)(DF)_T \, , \tag{A-5}$$

$$r_A = c(TV)(RF)(DF)_A \, . \tag{A-6}$$

In Equations A-4 to A-6, c is the mass concentration of DPM 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 DPM 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 the deposition model of Yu and Xu (1987).

The differential equations for $m_{iX}^{(i)}$, the mass of material component i in compartment X as a function of exposure time t, can be written as

Head (H)

$$\frac{dm_H^{(i)}}{dt} = r_H^{(i)} - \lambda_{HG}^{(i)} m_H^{(i)} - \lambda_{HB}^{(i)} m_H^{(i)} , \qquad \text{(A-7)}$$

Tracheobronchial (T)

$$\frac{dm_T^{(i)}}{dt} = r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{TG}^{(i)} m_T^{(i)} - \lambda_{TB}^{(i)} m_T^{(i)} , \qquad \text{(A-8)}$$

Alveolar (A)

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{AB}^{(i)} m_A^{(i)} , \qquad \text{(A-9)}$$

Lymph nodes (L)

$$\frac{dm_L^{(i)}}{dt} = \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{LB}^{(i)} m_L^{(i)} . \qquad \text{(A-10)}$$

Equation A-9 may also be written as

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_A^{(i)} m_A^{(i)} , \qquad \text{(A-11)}$$

where

$$\lambda_A^{(i)} = \lambda_{AT}^{(i)} + \lambda_{AL}^{(i)} + \lambda_{AB}^{(i)} . \qquad \text{(A-12)}$$

is the total clearance rate of material component i from the alveolar compartment. In Equations A-7 to A-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 DPM in compartment X is equal to

$$m_X = m_X^{(1)} + m_X^{(2)} + m_X^{(3)} \qquad \text{(A-13)}$$

The lung burdens of diesel soot (core) and organics are defined, respectively, as

$$m_{Lung}^{(1)} = m_T^{(1)} + m_A^{(1)} , \tag{A-14}$$

and

$$m_{Lung}^{(2)+(3)} = m_T^{(2)} + m_A^{(2)} + m_T^{(3)} + m_A^{(3)} . \tag{A-15}$$

Because the clearance of diesel soot from compartment T is much faster than from compartment A, $m_T^{(1)} < m_A^{(1)}$ a short time after exposure, Equation A-14 leads to

$$m_{Lung}^{(1)} \cong m_A^{(1)} . \tag{A-16}$$

Solution to Equations A-7 to A-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 A-7 to A-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 3, data have shown that when rats are exposed to DPM at high concentration for a prolonged period, 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 sequestering 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 A-7 to A-10 can be determined directly from experimental data on lung and lymph node burdens, and $\lambda_{AT}^{(i)}$ and $\lambda_{AB}^{(i)}$ from Equation A-12.

A.4. SOLUTIONS TO KINETIC EQUATIONS

Equation A-11 is a nonlinear differential equation of $m_A^{(i)}$ with known function of $\lambda_A^{(i)}$. For diesel soot, this equation becomes

$$\frac{dm_A^{(1)}}{dt} = r_A^{(1)} - \lambda_A^{(1)}(m_A)m_A^{(1)} . \qquad \text{(A-17)}$$

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 A-17 is then reduced to a differential equation with $m_A^{(1)}$ the only dependent variable.

The general solution to Equation A-17 for constant $r_A^{(1)}$ at any time, t, can be obtained by the separation of variables to give

$$\int_0^{m_A^{(1)}} \frac{dm_A^{(1)}}{r_A^{(1)} - \lambda_A^{(1)} m_A^{(1)}} = t . \qquad \text{(A-18)}$$

If $r_A^{(1)}$ is an arbitrary function of t, Equation A-17 needs to be solved numerically such as by a Runge-Kutta method. Once $m_A^{(1)}$ is found, the other kinetic equations A-7 to A-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)

$$m_H^{(i)} = r_H^{(i)}/\lambda_H^{(i)} + (m_{H0}^{(i)} - r_H^{(i)}/\lambda_H^{(i)}) \exp(-\lambda_H^{(i)} t) \qquad \text{(A-19)}$$

$$\text{where} \quad \lambda_H^{(i)} = \lambda_{HG}^{(i)} + \lambda_{HB}^{(i)} \qquad \text{(A-20)}$$

Tracheobronchial (T)

$$m_T^{(i)} = \exp\left(-\lambda_T^{(i)}\, t\right) \int_0^t \left(r_T^{(i)} + \lambda_{AT}^{(i)}\, m_A^{(i)} \right) \exp\left(\lambda_T^{(i)}\, t\right) dt + m_{T0}^{(i)} \qquad \text{(A-21)}$$

$$\text{where} \quad \lambda_T^{(i)} = \lambda_{TG}^{(i)} + \lambda_{TB}^{(i)} \qquad \text{(A-22)}$$

Lymph nodes (L)

$$m_L^{(i)} = \exp(-\lambda_{LB}^{(i)}\, t) \int_0^t \lambda_{AL}^{(i)} m_A^{(i)} \exp(\lambda_{LB}^{(i)}\, t)\, dt + m_{L0}^{(i)}) \qquad \text{(A-23)}$$

In Equations A-19 to A-23, $m_{XO}^{(i)}$ represents the value of $m_X^{(i)}$ at t = 0.

In the sections to follow, the methods of determining $r_H^{(i)}$, $r_T^{(i)}$ and $r_A^{(i)}$ or $(DF)_H$, $(DF)_T$, and $(DF)_A$ $r_H^{(DF)}$, $r_T^{(DF)}$ and $r_A^{(DF)}$ as well as the values of $\lambda_{XY}^{(i)}$ in the compartmental lung model are presented.

A.5. DETERMINATION OF DEPOSITION FRACTIONS

The mathematical models for determining the deposition fractions of DPM 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 DPM 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

$$\frac{d_e}{d_a} = \varphi^{-1/2}\left(\frac{C_a}{C_e}\right)^{1/2)}\left(\frac{\zeta}{\zeta_o}\right)^{1/2} \qquad \text{(A-24)}$$

where ζ is the bulk density of the particle in g/cm^3, ζ_0 = 1 g/cm^3; φ is the packing density, which is the ratio of the space actually occupied by primary particles in the envelope to the overall envelope volume; and C_x is the slip factor given by the expression:

$$C_x = 1 - 2\frac{\lambda}{d_x}\left[1.257 - 0.4 \exp -\left(\frac{0.55 d_x}{\lambda}\right)\right] \quad \text{(A-25)}$$

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 A-24 gives d_e/d_a = 1.35. In determining the deposition fraction of DPM, d_e is used for diffusion and interception according to the particle model.

A.5.1. Deposition in the Head

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 DPM:

For mouth breathing:

$$(DF)_{H,\,in} = 0, \; for \; d_a^2 \leq 3000 \quad \text{(A-26)}$$

$$(DF)_{H,\,in} = -1.117 + 0.324 \log(d_a^2 Q), \; for \; d_a^2 Q > 3000 \quad \text{(A-27)}$$

$$(DF)_{H,\,ex} = 0, \quad \text{(A-28)}$$

and for nose breathing:

$$(DF)_{H,\,in} = -0.014 + 0.023 \log(d_a^2 Q), \; for \; d_a^2 Q \leq 337 \quad \text{(A-29)}$$

$$(DF)_{H,\ in} = -0.959 + 0.397 \log(d_a^2 Q),\ for\ d_a^2 Q > 337 \quad \text{(A-30)}$$

$$(DF)_{H,\ ex} = 0.003 + 0.033 \log(d_a^2 Q),\ for\ d_a^2 Q \leq 215 \quad \text{(A-31)}$$

$$(DF)_{H,\ ex} = -0.851 + 0.399 \log(d_a^2 Q),\ for\ d_a^2 Q > 215 \quad \text{(A-32)}$$

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 A-26 through A-32 are transformed into the following form:
For mouth breathing:

$$(DF)_{H,\ in} = 0,\ for\ d_a^2 Q \leq 3000 \quad \text{(A-33)}$$

and for nose breathing:

$$(DF)_{H,\ in} = -1.117 + 0.972\ logK + 0.324 \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \quad \text{(A-34)}$$

$$(DF)_{H,\ ex} = 0. \quad \text{(A-35)}$$

$$(DF)_{H,\ in} = -0.014 + 0.690 \log K + 0.023 \log(d_a^2 Q),\ for\ d_a^2 Q \leq 337 \quad \text{(A-36)}$$

$$(DF)_{H,\ in} = -0.959 + 1.191 \log K + 0.397 \log (d_a^2 Q),\ \text{for}\ d_a^2 Q > 337 \quad \text{(A-37)}$$

$$(DF)_{H,\ ex} = 0.003 + 0.099 \log K + 0.033 \log(d_a^2 Q),\ \text{for}\ d_a^2 Q \leq 215 \quad \text{(A-38)}$$

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.

$$(DF)_{H,\,ex} = 0.851 + 1.197 \log K + 0.399 \log(d_a^2 Q), \text{ for } d_a^2 Q > 215 \qquad \text{(A-39)}$$

For rats, the following empirical equations are used for deposition prediction of DPM in the nose:

$$(DF)_{H,\,in} = (DF)_{H,\,ex} = 0.046 + 0.009 \log(d_a^2 Q), \text{ for } d_a^2 Q \leq 13.33 \qquad \text{(A-40)}$$

A.5.2. Deposition in the Tracheobronchial and Alveolar Regions

The deposition model adopted for DPM is the one previously developed for monodisperse (Yu, 1978) and polydisperse spherical aerosols (Diu and Yu, 1983). In the model,

$$(DF)_{H,\,in} = (DF)_{H,\,ex} = -0.522 + 0.514 \log(d_a^2 Q), \text{ for } d_a^2 Q > 13.33 \qquad \text{(A-41)}$$

the branching airways are viewed as a chamber model shaped like a trumpet (Figure A-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. 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:

$$\beta(A_1 + A_2)\frac{\partial c}{\partial x} + Q\frac{\partial c}{\partial x} = -Qc\eta \qquad \text{(A-42)}$$

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:

$$\beta = 1 + \frac{V_t}{V_l} \qquad \text{(A-43)}$$

and Q is the air flow rate, varying with x and t according to the relation

$$\frac{Q}{Q_o} = 1 - \frac{V_x}{V_l} \tag{A-44}$$

where Q_0 is the air flow rate at x = 0. In Equations A-43 and A-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 A-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

$$DF = \int_{t_1}^{t_2}\int_{x_1}^{x_2} Qc\eta\, dx dt \tag{A-45}$$

For diesel particles, η is the sum of those due to the individual deposition mechanisms described above, i.e.,

where η_I, η_S, η_P, and η_D are, respectively, the deposition efficiencies per unit length of the

$$\eta = \eta_I + \eta_S + \eta_P + \eta_D \tag{A-46}$$

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:

$$\eta_I = \frac{0.768}{L}(St)\theta. \tag{A-47}$$

$$\eta_S = \frac{2}{\pi L}[2\epsilon\sqrt{1-\epsilon^{(2/3)}} - \epsilon^{1/3}\sqrt{1-\epsilon^{2/3}} + \sin^{-1}\epsilon^{1/3}] \tag{A-48}$$

$$\eta_P = \frac{4}{3\pi L}(\Gamma - \frac{\Gamma^3}{32}) \tag{A-49}$$

$$\eta_D = \frac{1}{L}[1-0.819\exp(-14.63\Delta) - 0.0976\exp(-89.22\Delta) - 0.0325\exp(-228\Delta) - 0.0509\exp(-125\Delta^{2/3})] \quad \text{(A-50)}$$

$$\eta_D = \frac{4}{L}\Delta^{1/2}(1 - 0.444\Delta^{1/2}) \quad \text{(A-51)}$$

for Reynolds numbers of the flow smaller than 2000, and 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 $D = C_c kT/(3\pi\mu d_a)$ is the diffusion coefficient with k denoting the Boltzmann constant and T the absolute temperature. In the deposition model, it is also assumed that η_I and $\eta_P = 0$ for expiration, while η_D and η_S have the same expressions for both inspiration and expiration.

During the pause, only diffusion and sedimentation are present. The combined deposition efficiency in the airway, E, is equal to:

$$E = 1 - (1 - E_S)(1 - E_D) . \quad \text{(A-52)}$$

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

$$E_D = 1 - \sum_{i=1}^{3} \frac{4}{\alpha_i} \exp(-\alpha_i^2\tau_D)(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2}) \exp\left[- \frac{4\tau_D^{1/2}}{\pi^{1/2}(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2})} \right] \quad \text{(A-53)}$$

where $\tau_D = D\tau/R^2$ in which τ is the pause time and α_1, α_2, and α_3 are the first three roots of the equation:

$$J_o(\alpha) = 0 . \quad \text{(A-54)}$$

in which J_o is the Bessel function of the zero$_{th}$ order, and:

$$E_S = 1.1094\tau_S - 0.1604\tau_S^2 \text{ for } 0 < \tau_S \leq 1. \quad \text{(A-55)}$$

and

$$E_S = 1 - 0.0069\tau_S^{-1} - 0.0859\tau_S^{-2} - 0.0582\tau_S^{-3}, \quad for\ \tau_S > 1, \tag{A-56}$$

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.

A.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.

A.5.3.1. *Lung Model for Rats*

Morphometric data on the lung airways of rats were reported by Schum and Yeh (1979). Table A-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.

A.5.3.2. *Lung Model for Human Adults*

The lung model of mature human adults used in the deposition calculation of DPM 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 A-2 presents the morphometric data of the airways of Weibel's model adjusted to a total lung volume of 3000 cm^3.

A.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.

A.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

$$N_i(t) = 2^i, \qquad \text{for } 0 \le i \le 20 \tag{A-57}$$

$$\begin{cases} N_{21}(t) = N_r(t), \\ N_{22}(t) = N_{23}(t) = 0. \end{cases} \qquad \text{for } N_r(t) \le 2^{21} \tag{A-58}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = N_r(t) - 2^{21}, \\ N_{23}(t) = 0, \end{cases} \qquad \text{for } 2^{21} < N_r(t) \le 2^{22} \tag{A-59}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = 2^{22}, \\ N_{23}(t) = N_r(t) - 2^{21} - 2^{22} \end{cases} \qquad \text{for } N_r(t) > 2^{21} + 2^{22}, \tag{A-60}$$

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

Thus, $N_r(t)$ increases from approximately 1.5 million at birth to 15 million at 8 years of age and

$$N_r(t) = \begin{cases} 2.036 \times 10^7 (1 - 0.926 e^{-0.15t}), & t \le 8 \\ 1.468 \times 10^7, & t > 8 \end{cases} \tag{A-61}$$

remains nearly constant thereafter. Equations A-58 to A-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:

$$N_A(t) = 2.985 \times 10^8(1 - 0.919e^{-0.45t}) \quad \text{(A-62)}$$

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.

A.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

$$LV(t) = 0.959 \times 10^5(1 - 0.998e^{-0.002t}) \quad (cm^3). \quad \text{(A-63)}$$

The growth patterns of the bronchial airways are determined by the following equations

$$D_i(t) - D_{iw} = \alpha_i[H(t) - H(25)], \quad \text{(A-64)}$$

$$L_i(t) - L_{iw} = \beta_i[H(t) - H(25)], \quad \text{(A-65)}$$

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

$$\alpha_i = 3.26 \times 10^{-2}\exp[-1.183\ (i+1)^{0.5}] \quad \text{(A-66)}$$

$$\beta_i = 1.05 \times 10^{-6}\ \exp\ [10.1]\ (i+1)^{-0.2}] \quad \text{(A-67)}$$

and H(t) is the body height, which varies with age t in the form

$$H(t) = 1.82 \times 10^2(1 - 0.725e^{-0.14t}) \ (cm). \tag{A-68}$$

For the growth patterns of the airways in the alveolar region, it is assumed that

$$\frac{D_i}{D_{iw}} = \frac{L_i}{L_{iw}} = \frac{D_a}{D_{aw}} = f(t), \quad for \ 17 \le i \le 23 \tag{A-69}$$

where D_a is the diameter of an alveolus at age t, $D_{aw} = 0.0288$ cm is the alveolar diameter for adults in accordance with Weibel's model, and f(t) is a function determined from

$$f(t) = \sqrt[3]{\frac{\{LV(t) - \sum_{i=0}^{16} \frac{\pi}{4} D_i^2(t) \ L_i(t) N_i(t)\}}{\{\sum_{i=17}^{23} \frac{\pi}{4} D_{iw}^2 L_{iw} N_i(t) + \frac{5\pi}{36} D_{aw}^3 N_A(t)\}}} \tag{A-70}$$

A.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:

$$\lambda_{HG}^{(i)} = 1.73 \ (i = 1,2,3) \tag{A-71}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(i)} = 0.00018 \tag{A-72}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \tag{A-73}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \tag{A-74}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{A-75}$$

$$\lambda_{AL}^{(1)} = 0.00068\ [1 - \exp(-0.046 m_A^{1.62})] \tag{A-76}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4}\lambda_{AB}^{(i)} \qquad (i = 2,3) \tag{A-77}$$

$$\lambda_{AT}^{(i)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) \quad (i = 1,2,3) \tag{A-78}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AT}^{(1)} + \lambda_{AB}^{(1)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00086 \tag{A-79}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_a^{1.62}) + 0.0161 \tag{A-80}$$

$$\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) + 15.7 \tag{A-81}$$

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 DPM in humans are not available. To estimate the lung burden of DPM for human exposure, it is necessary to extrapolate the transport rates $\lambda_{XY}^{(i)}$ from rats to humans. For organics, it is assumed 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).

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 A-79 that $\lambda_A^{(1)}$ for rats can be written in the form

$$\lambda_A^{(1)} = a \exp(-bm_A^c) + d \qquad \text{(A-82)}$$

where a, b, c, and d are constants. The right-hand side of Equation A-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

$$\lambda_A^{(1)} = \frac{a}{P} \exp[-b(m_A/S)^c] + d \qquad \text{(A-83)}$$

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 A-83 implies that rats and humans have equivalent amounts of biological response in the lung to the same specific surface dose of inhaled DPM.

From the data of Bailey et al. (1982), a value of $\lambda_A^{(1)} = 0.00169$ day^{-1} is obtained for humans at low lung burdens leading to P = 14.4. A value for S of 148 is reported 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, the model assumes 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 A-3.

The equations for other transport rates that have a lung-burden-dependent component are extrapolated from rats to humans in a similar manner. The following lists the values of $\lambda_{XY}^{(1)}$ (in day^{-1}) for humans used in the present model calculation:

$$\lambda_{HG}^{(1)} = 1.73 \quad (i = 1,2,3) \qquad \text{(A-84)}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(1)} = 0.00018 \tag{A-85}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \tag{A-86}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \tag{A-87}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{A-88}$$

$$\lambda_{AL}^{(1)} = 0.00068 \{1 - 0.0694 \exp[-0.046(m_A/S)^{1.62}]\} \tag{A-89}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4} \lambda_{AB}^{(i)} \qquad (i = 2, 3) \tag{A-90}$$

$$\lambda_{AT}^{(i)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} \quad (i = 1, 2, 3) \tag{A-91}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AB}^{(1)} + \lambda_{AT}^{(1)} = 0.0694 \{0.012 \exp[-0.11(m_A/S)^{1.76}]\} + 0.00086 \tag{A-92}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.0694\{0.012 \exp[-0.11(m_A/A)^{1.76}] + 0.00068 \exp[-0.046(m_A/S)^{1.76}]\} + 0.016 \tag{A-93, A-94}$$

A.7. RESULTS

A.7.1. Simulation of Rat Experiments

To test the accuracy of the model, simulation results are obtained on the retention of DPM 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.

$$\text{Minute volume} = 0.9W \ (\text{cm}^3/\text{min}) \tag{A-95}$$

$$\text{Respiratory frequency} = 475W^{-0.3} \ (1/\text{min}) \tag{A-96}$$

where W is the body weight (in grams) as determined from the equation

$$W = 5+537T/(100+T), \text{ for } T \geq 56 \text{ days} \tag{A-97}$$

in which T is the age of the rat measured in days.

Equation A-95 was obtained from the data of Mauderly (1986) for rats ranging in age from 3 mo to 2 years old; Equation A-96 was obtained from the data of Strom et al. (1988); and Equation A-97 was determined from the best fit of the experimental deposition data. Figures A-3 and A-4 show the calculated lung burden of diesel soot ($m_A^{(1)} + m_T^{(1)}$) and lymph node burden, respectively, for the experiment by Strom et al. (1988) using animals exposed to DPM 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 A-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 $m_A \sim 0$. Figure A-5 shows that the calculated retention is in excellent agreement with the experimental data obtained by Sun et al. (1984).

A.7.2. Predicted Burdens in Humans

Selected results of lung burden predictions in humans are shown in Figures A-6 to A-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 A-6 and A-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 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 A-8 and A-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.

A.8. PARAMETRIC STUDY OF THE MODEL

The deposition and clearance model of DPM 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.

A.8.1. Effect of Ventilation Conditions

The changes in lung burden due to variations in tidal volume and respiratory frequency are depicted in Figures A-10 and A-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 DPM inhaled. The mode of breathing has only a minor effect on lung burden because switching from nose breathing does not produce any appreciable change in the amount of particle intake into the lung (Yu and Xu, 1987). All lung burden results presented in this report are for nose breathing.

A.8.2. Effect of Transport Rates

Transport rates have an obvious effect on the retention of DPM 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 A-12 and A-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 A-12), while just the opposite occurred when the multiple of $\lambda_A^{(2)}$ was increased (Figure A-13).

A.9. OPERATIONAL DERIVATION OF HUMAN EQUIVALENT CONCENTRATIONS (HECs)

The model of Yu et al. (1991) is ordered into two parts; one part parameterized on the physiology and anatomy of a 300 g rat and the other part parameterized on the physiology and anatomy of a 25 year old human male. The sequence of steps taken to calculate the human equivalent continuous concentrations (the HECs), outlined in Table A-4, were as follows:

- The exposure scenario of the rats was entered into the rat portion of the model and the model ran to obtain the output of lung burden in mg DPM/ rat lung at the time of the sacrifice of the rats.
- The output of mg DPM/ rat lung was normalized to mg DPM/ cm^2 of rat lung tissue based on a total pulmonary surface area of 4090 cm^2.

- The normalized rat lung burdens were used to calculate the corresponding lung burden based on the pulmonary surface area of 627,000 cm^2. This operation yielded mg DPM / lung of a 25 year old human male.
- Various air concentrations were run in an iterative fashion with the human portion of the model under a continuous exposure scenario of 24 hrs/day, 7d/wk for 70 years with ventilatory parameters set at 0.926 L for tidal volume and 15 breaths per minute as the respiratory frequency to yield a total daily pulmonary volume of 20 m^3. This was continued until the output (mg DPM/lung) was matched to the mg DPM /human lung obtained from the normalized rat lung burden; the concentration from the model that matched this lung burden was termed the human equivalent continuous concentration, the HEC. The human modeling runs did not consider the preadult status of airway and alveoli number discussed above but rather were ran for 1 to 70 years with adult (25 years of age) parameters mentioned above.

These HEC values address kinetic issues of DPM deposition and retention in the lung by humans. As noted above, these values do not reflect the kinetic variability that may exist in the human population exposed to DPM which includes men and women, young and old. However, the limited parametric analysis of the model clearly shows variability of those parameters most determinative in humans (e.g., tidal volume, respiration rate, and rates of clearance of particles from the airways) were mirrored in the corresponding output of the model (lung burden of DPM). One interpretation of this parallel in parameter-output is that the variability in the physiological characteristics of humans reflects the variability in the model such that, for example, a small tidal volume would be reflected with a decreased lung burden of DPM. Variability among humans of these key parameters such as tidal volume do vary but within an order of magnitude. This would mean that the DPM dose received by different individuals in the population from the same concentration would indeed vary within the extremes of these determinative parameters.

Table A-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 A-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

Table A-3. Ratio of pulmonary surface areas between humansand 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

Table A-4. Human equivalent continuous concentrations (HECs) calculated with the model of Yu et al. (1991) from long-term repeated exposure rat studies of DPM exposure

Study	Exposure conditions[a]	Rat exposure concs (mg/m^3)	mg DPM/ rat lung (modeled)[b]	mg DPM/cm^2 rat&human lung[b,c]	mg DPM/ human lung[c]	HEC (mg/m^3)[c]
Mauderly et al., 1987a	7 h/day, 5 days/wk, 130 wk[d]	0.35	0.28	6.85E-5	43	0.038
Mauderly et al., 1987a	7 h/day, 5 days/wk, 130 wk	3.47	20.23	4.95E-3	3101	1.375
Mauderly et al., 1987a	7 h/day, 5 days/wk, 130 wk	7.08	44.52	1.09E-2	6825	3.05
Ishinishi et al., 1988 (LD[e])	16 h/day, 6 days/wk, 130 wk	0.11	0.24	5.87E-5	37	0.032
Ishinishi et al., 1988 (LD)	16 h/day, 6 days/wk, 130 wk	0.41	1.00	2.45E-4	153	0.128
Ishinishi et al., 1988 (LD)	16 h/day, 6 days/wk, 130 wk	1.18	18.45	4.51E-3	2828	1.25
Ishinishi et al., 1988 (LD)	16 h/day, 6 days/wk, 130 wk	2.32	39.89	9.75E-3	6115	2.75
Ishinishi et al., 1988 (HD)	16 h/day, 6 days/wk, 130 wk	0.46	1.15	2.81E-4	176	0.144
Ishinishi et al., 1988 (HD)	16 h/day, 6 days/wk, 130 wk	0.96	12.94	3.16E-3	1984	0.883
Ishinishi et al., 1988 (HD)	16 h/day, 6 days/wk, 130 wk	1.84	31.22	7.63E-3	4786	2.15
Ishinishi et al., 1988 (HD)	16 h/day, 6 days/wk, 130 wk	3.72	64.67	1.58E-2	9914	4.4
Nikula et al., 1995	16 h/day, 5 days/wk, 100 wk	2.44	28.64	7.00E-3	4391	1.95
Nikula et al., 1995	16 h/day, 5 days/wk, 100 wk	6.3	76.15	1.86E-2	11674	5.1
Heinrich et al., 1995	18 h/day, 5 days/wk, 104 wk	0.84	3.83	9.4E-4	587	0.33
Heinrich et al., 1995	18 h/day, 5 days/wk, 104 wk	2.5	34.4	8.4E-3	5274	2.35
Heinrich et al., 1995	18 h/day, 5 days/wk, 104 wk	6.98	97.8	2.4E-2	14993	6.7

[a] These are entered into the program as hrs/day, days/week for the total number of weeks exposed and the last week of exposure before evaluation (as this would affect clearance). The parameters for the rat were based on a body weight which was set in the program at 300g.

[b] These values were obtained with the rat portion of the model and are noted as lung burden, in mg DPM /lung of a 300 g rat, at the final week of the exposure scenario. These outputs were then normalized to cm^2 of the rat lung, at 4090 cm^2 total (Xu and Yu, 1987).

[c] Preparatory to using the human portion of the model, the mg DPM/cm^2 value from above was used to project the mg DPM that would be present in the adult human lung based on a total lung surface area of 627,000 cm^2 (Xu and Yu, 1987). Various air concentrations were then entered into the human model as 70 years continuous exposure scenarios and ran iteratively until the output (in mg DPM / lung at age 70) matched this mg DPM/human lung, i.e., the total lung burden. This matching air concentration is, by definition, the human equivalent continuous concentration (HEC).

[d] weeks = (months of exposure) × 4.33.

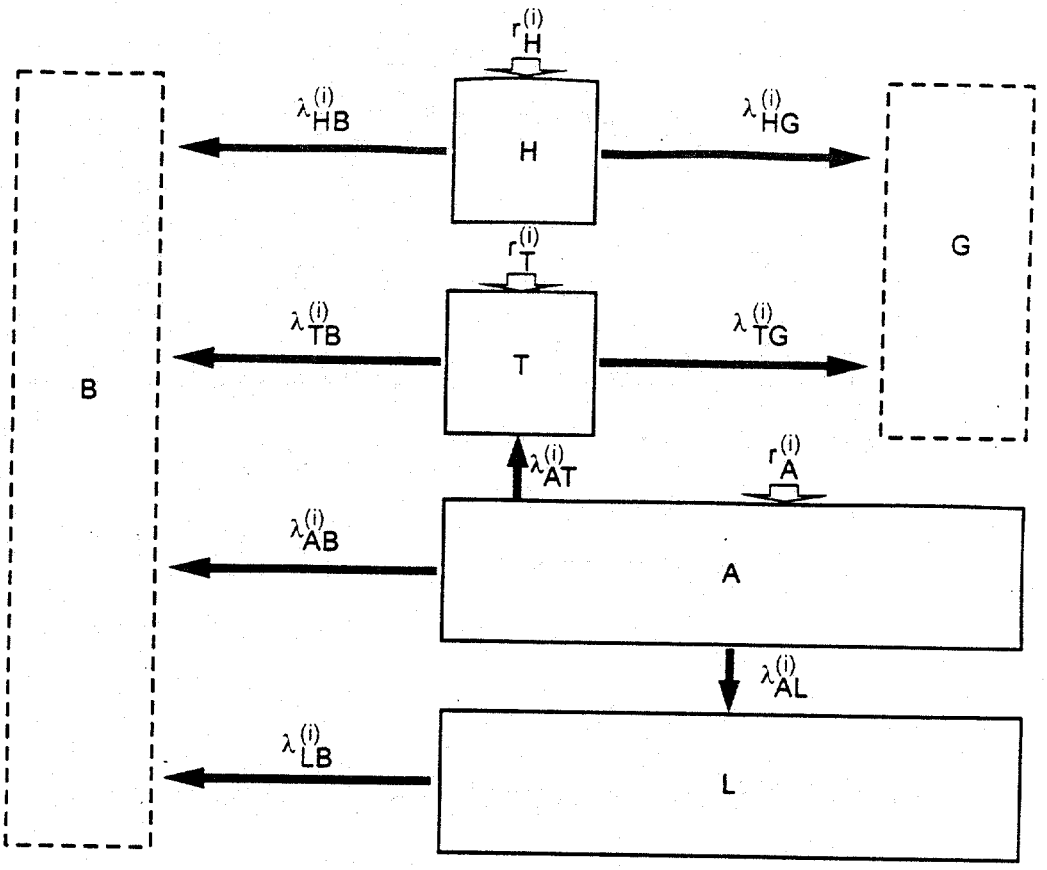

Figure A-1. Compartmental model of DPM retention.

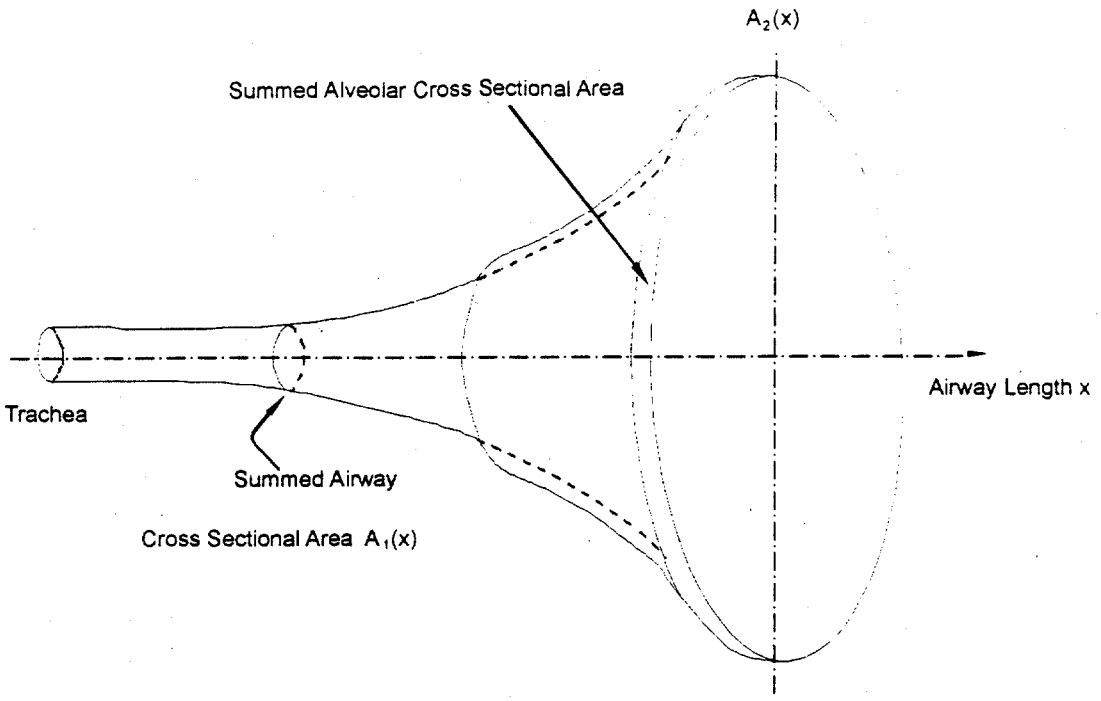

Figure A-2. Trumpet model of lung airways.

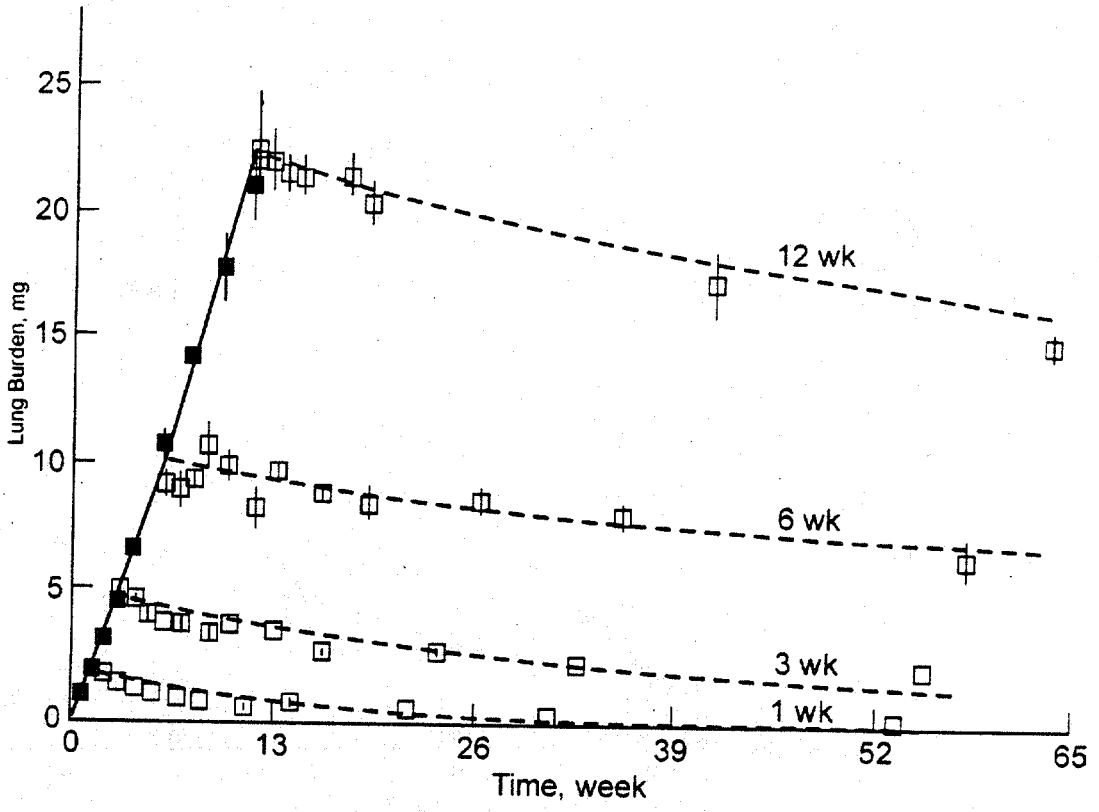

Figure A-3. The experimental and predicted lung burdens of rats to DPM at a solid and dashed concentration of 0.6 mg/m^3 for different exposure spans. Lines are, respectively, the predicted burdens during exposure and post-exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

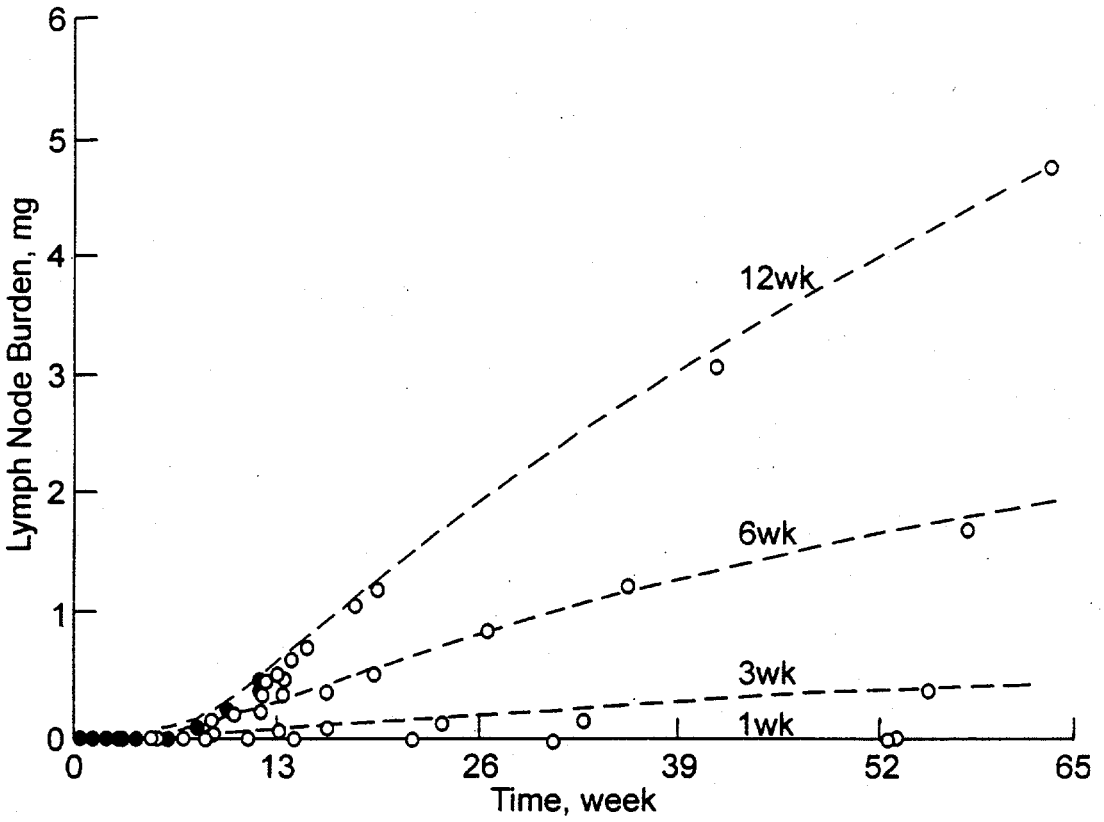

Figure A-4. Experimental and predicted lymph node burdens of rats exposed to CEPs at a concentration of 6.0 mg/m^3 for different exposure spans. The solid and dashed lines are, respectively, the predicted burdens during exposure and post-exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

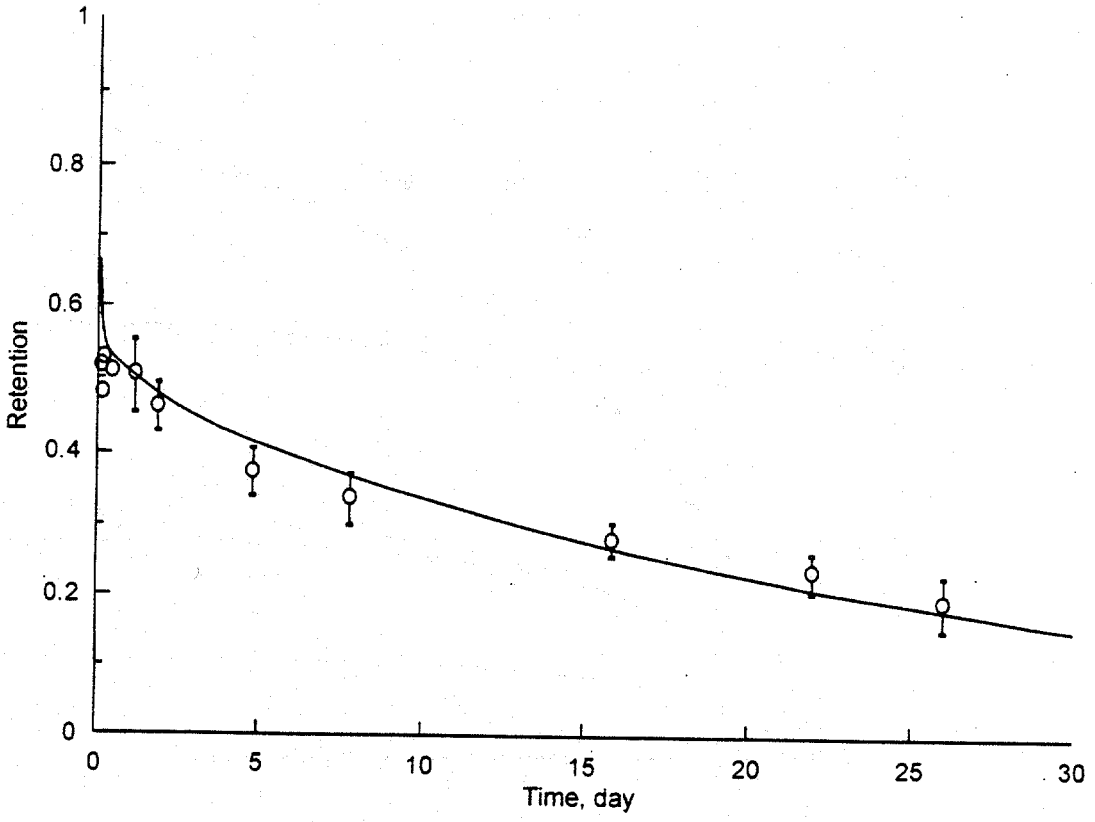

Figure A-5. Comparison between the calculated lung retention (solid line) and the experimental data obtained by Sun et al. (1984) for the particle-associated BaP in rats.

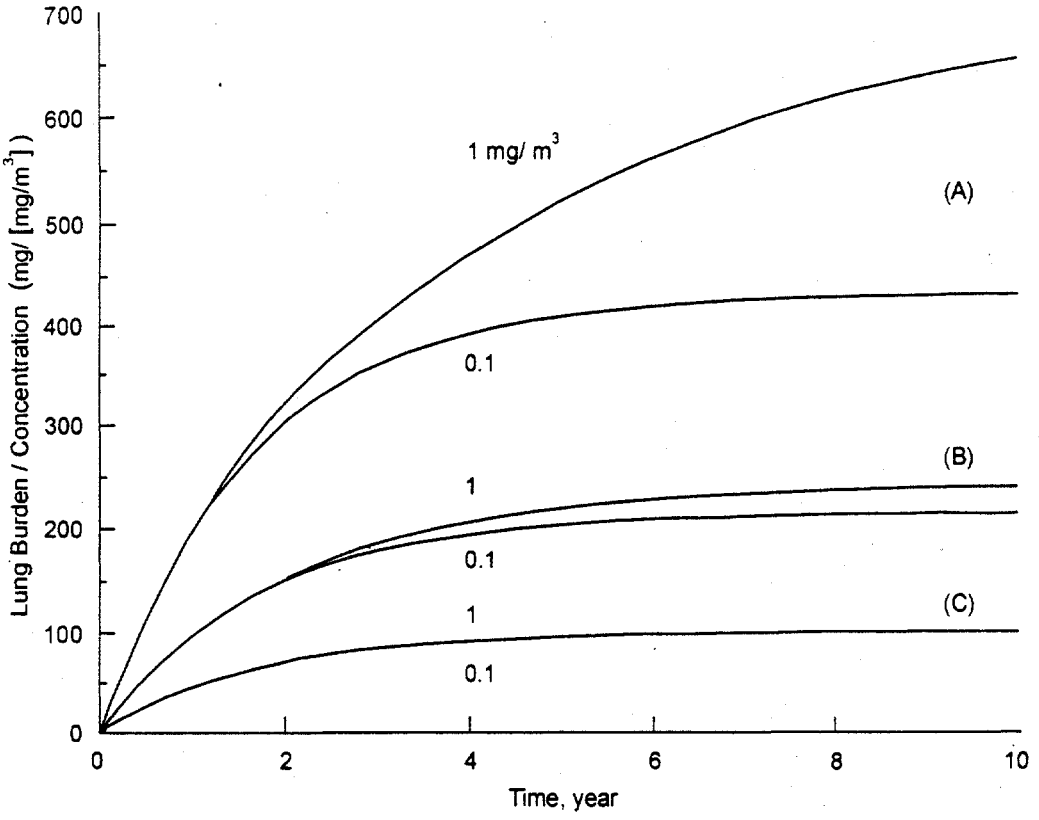

Figure A-6. Calculated lung burdens of diesel soot per unit exposure concentration in human adults exposed continuously to DPM at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

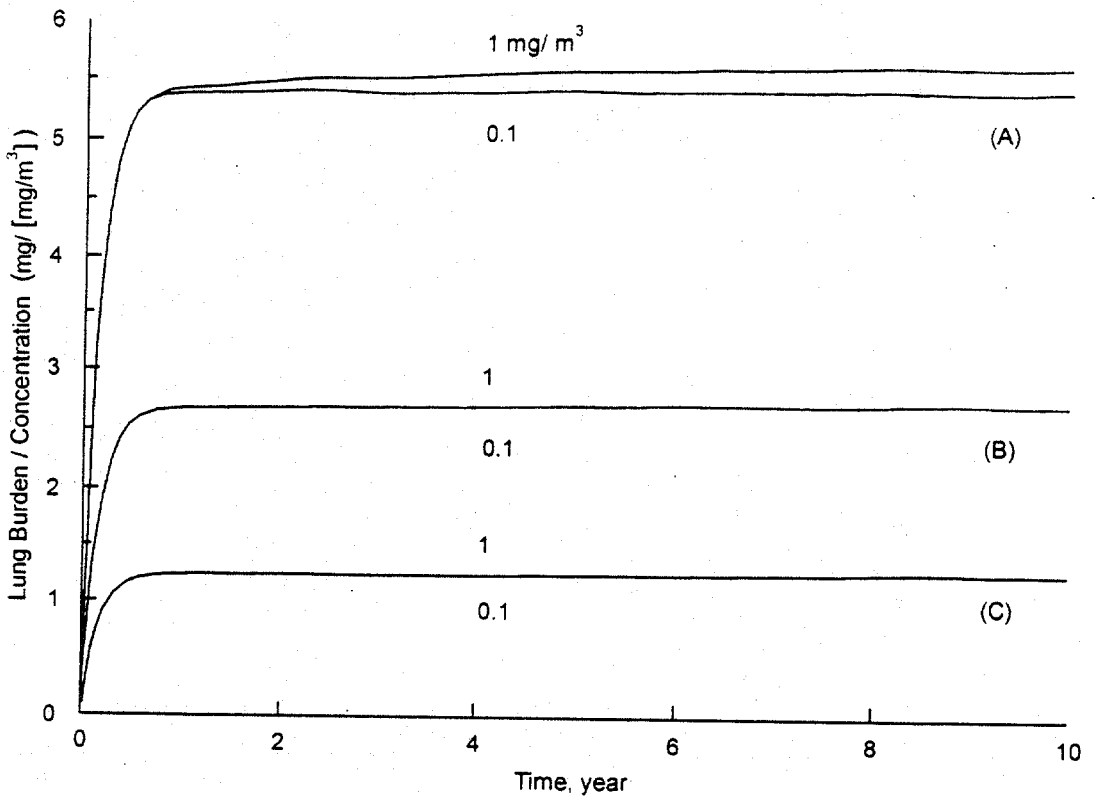

Figure A-7. Calculated lung burdens of the particle-associated organics per unit exposure concentration in human adults exposed continuously to DPM at two different concentrations of 0.1 and 1.0 mg/m^3. Exposure patterns are (a) 24 h/day and 7 days/week, (b) 12 h/day and 7 days/week, and (c) 8 h/day and 5 days/week.

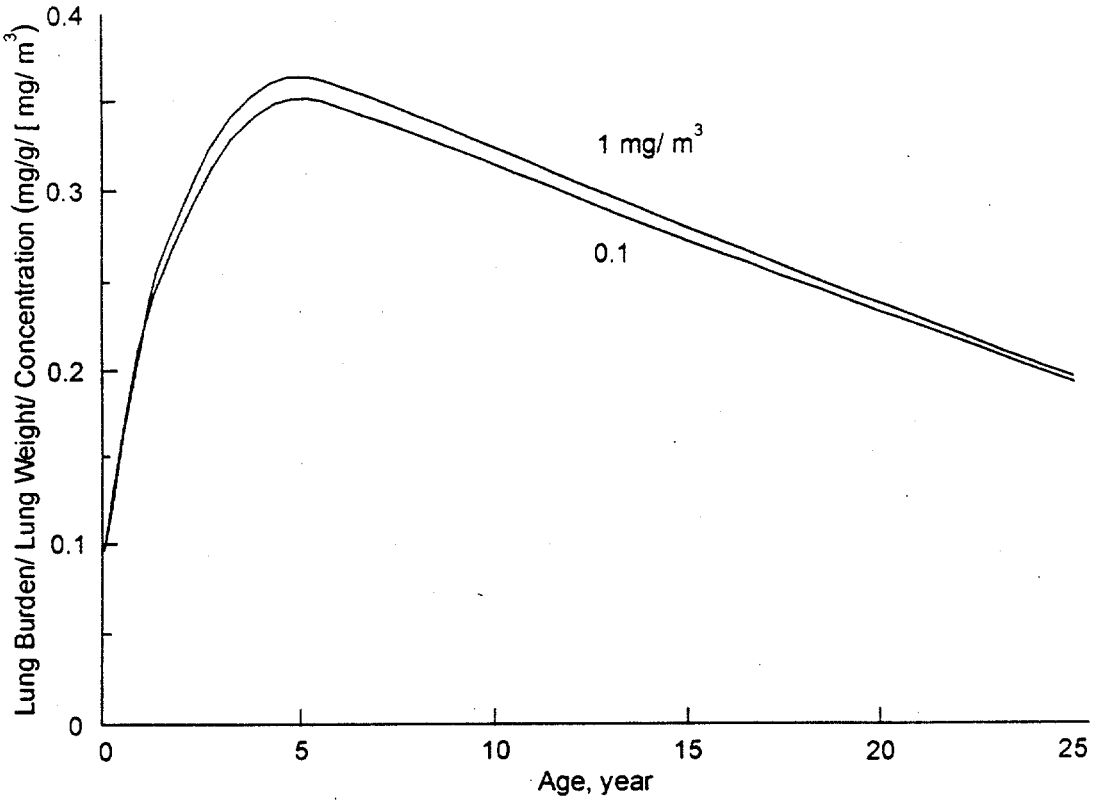

Figure A-8. Calculated lung burdens of diesel soot per gram of lung per unit exposure concentration in humans of different ages exposed continuously for 1 year to DPM of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.

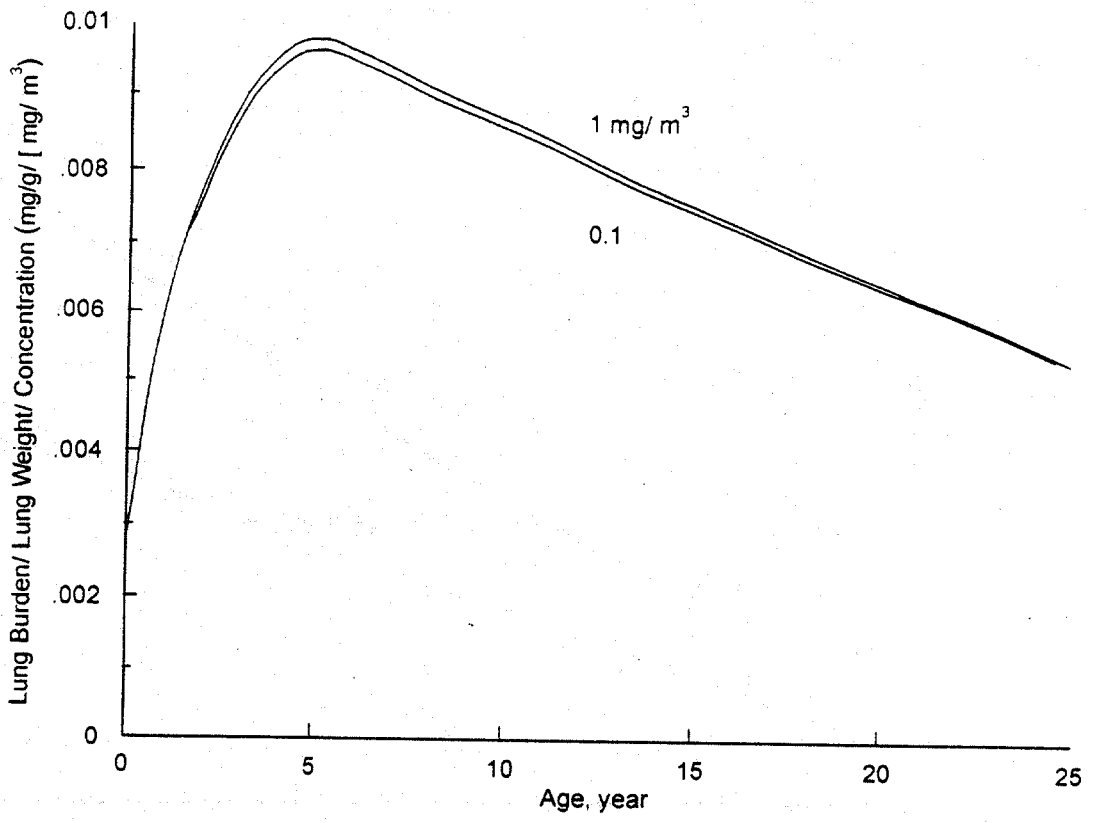

Figure A-9. Calculated burdens of the particle-associated organics per gram of lung per unit exposure concentration in humans of different ages exposed continuously for 1 year to DPM of two different concentrations of 0.1 and 1.0 mg/m^3 for 7 days/week and 24 h daily.

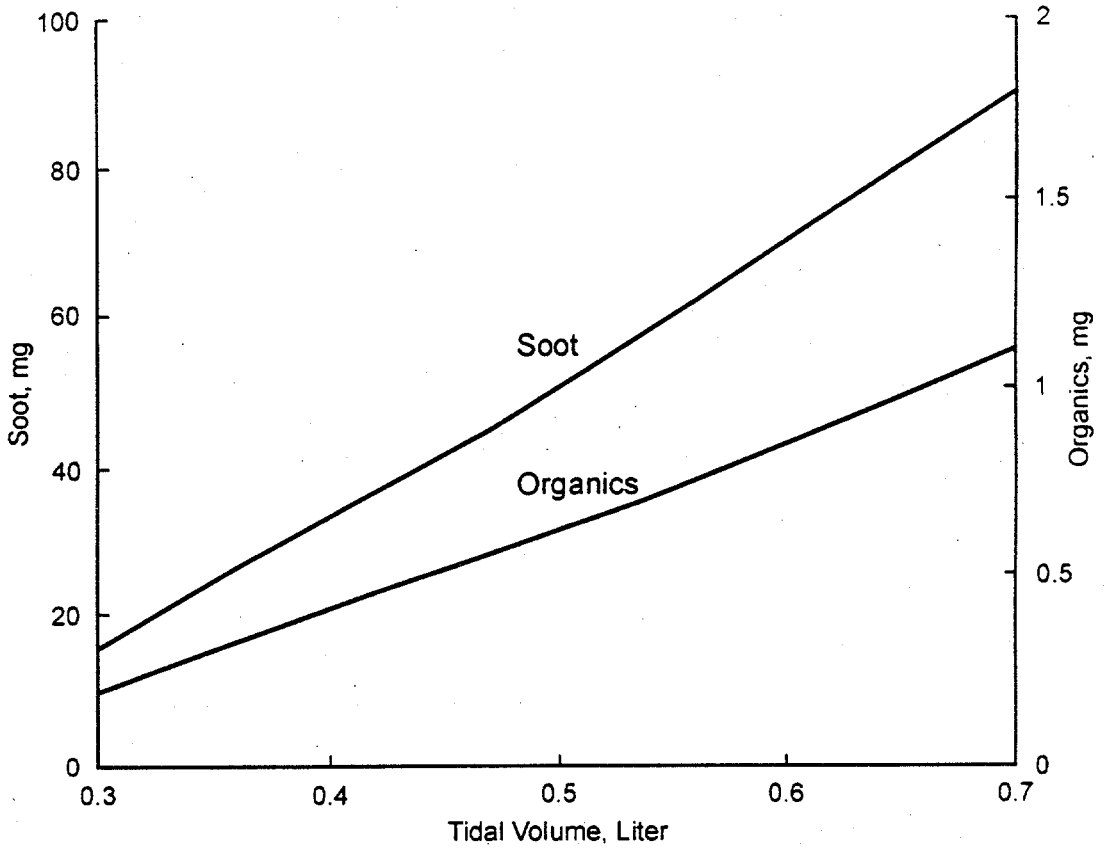

Figure A-10. Calculated lung burdens in human adults versus tidal volume in liters for exposure to DPM at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) respiratory frequency = 14 min^{-1}; and (c) lung volume = 3000 cm^3.

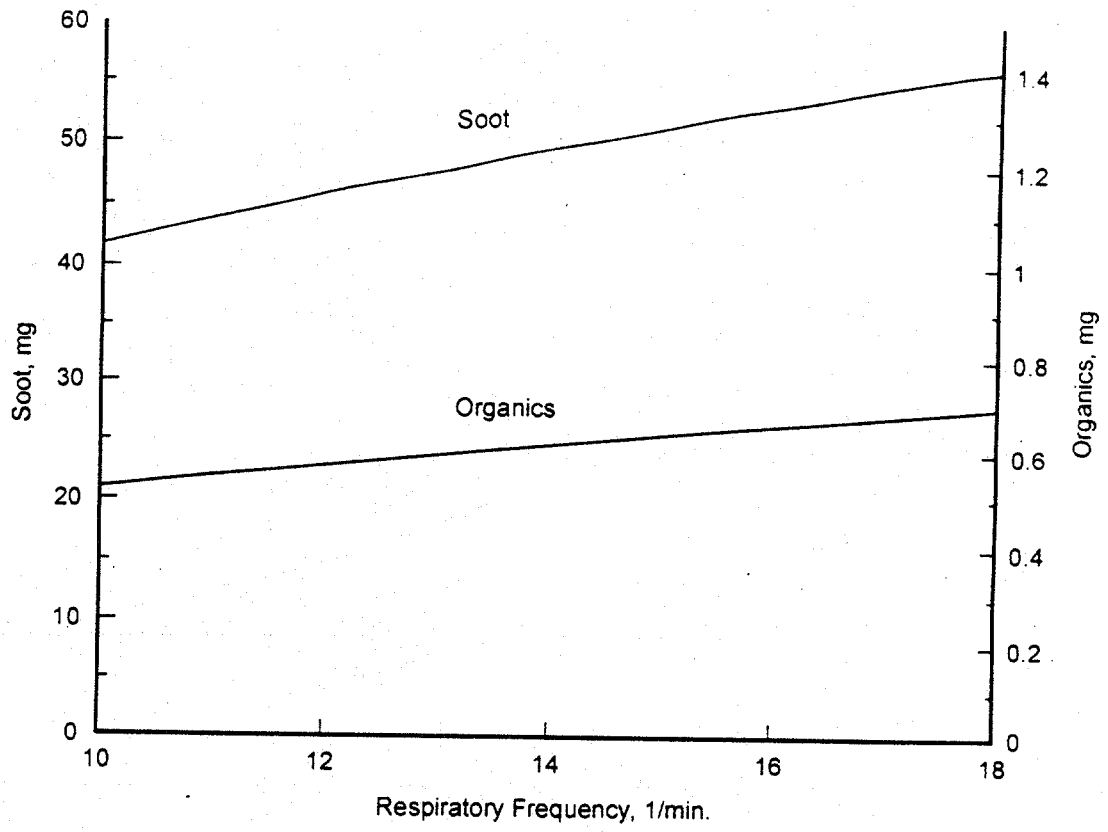

Figure A-11. Calculated lung burdens in human adults versus respiratory frequency in *bpm* for exposure to DPM at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, and (c) lung volume = 3200 cm^3.

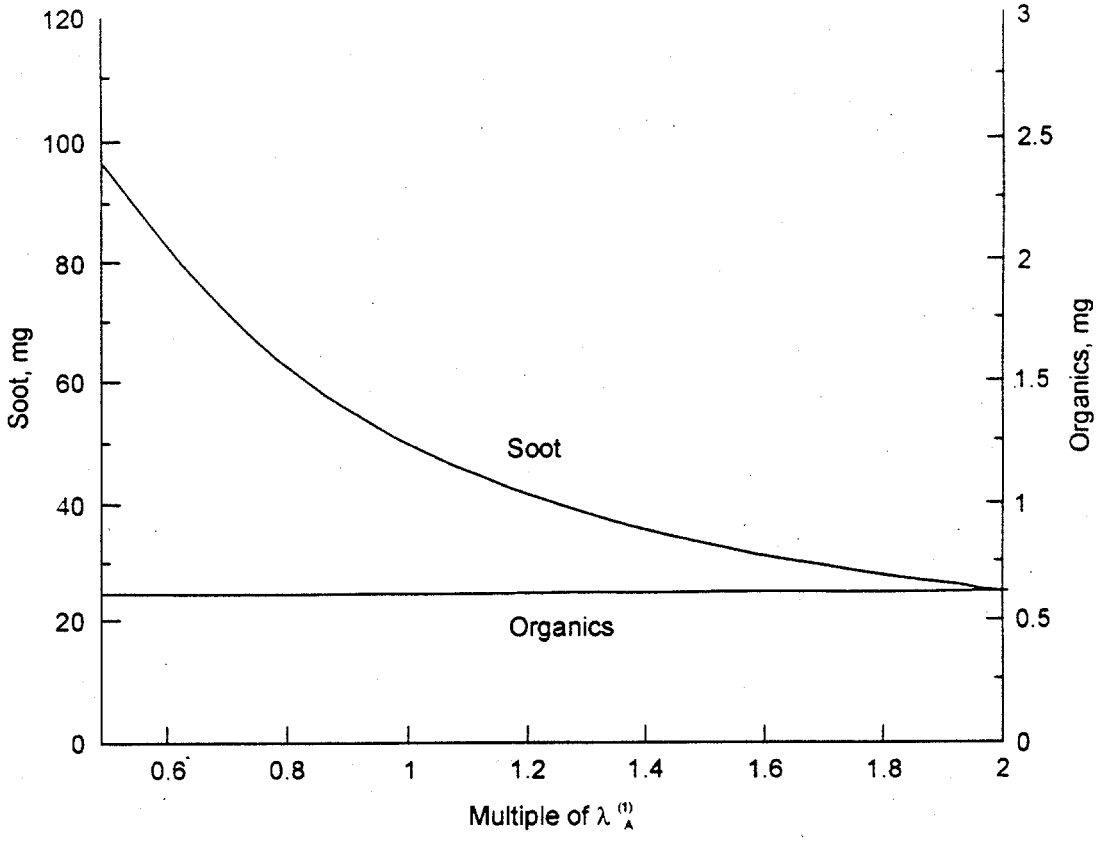

Figure A-12. Calculated lung burdens in human adults versus multiple of $\lambda_A^{(1)}$ for exposure to DPM at 0.1 mg/m$_3$ for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}; and (c) lung volume = 3200 cm^3.

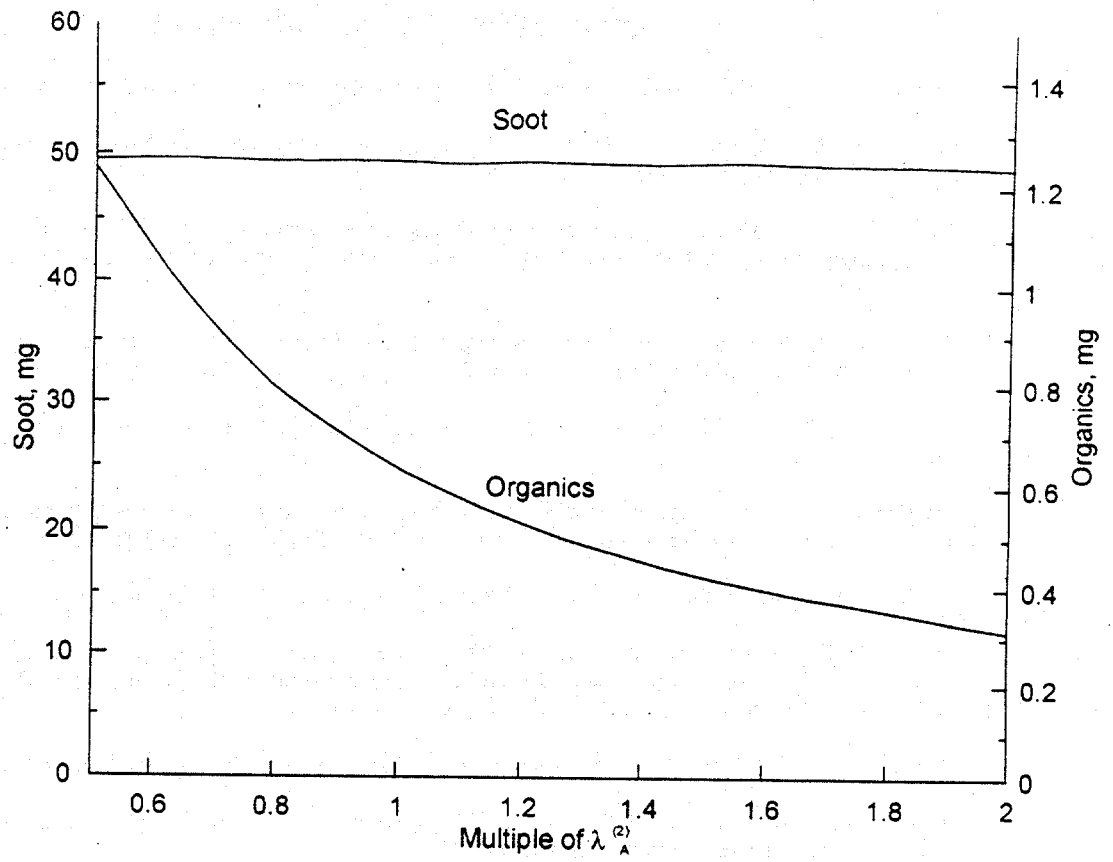

Figure A-13. Calculated lung burdens in human adults versus multiple of $\lambda_A^{(1)}$ for exposure to DPM at 0.1 mg/m^3 for 10 years at 7 days/week and 24 h daily. Parameters used in the calculation are: (a) MMAD=0.2 μm σ_g=2.3, f_2=0.1, f_3=0.1; (b) tidal volume = 500 cm^3, respiratory frequency = 14 min^{-1}; and (c) lung volume = 3200 cm^3.

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Appendix B

Benchmark Concentration Analysis of Diesel Data

B-1. INTRODUCTION TO BENCHMARK

The benchmark dose or benchmark concentration approach, hereafter referred to as the BMC approach, is an alternate to the N/LOAEL option for deriving effect levels. The BMC is currently undergoing extensive consideration by the Agency with promulgation of software and guidelines for application of this methodology (U.S. EPA, 2000). The BMC approach involves fitting a dose-response function to dose and effect information from a single study to derive the best fit of those data. This "best fit"is statistically termed the maximum likelihood estimate but is referred to in the benchmark terminology as the BMC curve. The curve defining the corresponding lower 95% confidence limit of this "best fit"estimate is termed the BMCL curve. This BMCL curve is used to predict the dose that will result in a level of response that is defined *a priori* as the benchmark response "x", $BMCL_x$. In the analyses below, for example, the benchmark response for a 10% increase in incidence[1] of chronic inflammation is defined as a $BMCL_{10}$; the corresponding 10% increase as determined from the BMC curve would be termed the BMC_{10}. This $BMCL_{10}$ would be derived by first using the data and the programs to determine the BMC and BMCL curves. The concentration corresponding to a 10% increase in incidence would then be determined directly from the BMCL. The $BMCL_{10}$ then would be used as the representative value for the effect level or point of departure in the dose-response assessment.

The latest version of the Agency Benchmark Dose Software (BMDS Version 1.2; U.S. EPA, 2000) was used to analyze data on chronic inflammation and pulmonary histopathology present in the chronic studies that were amenable to benchmark analysis. At this time, the Agency BMDS offers sixteen different models total that are appropriate for the analysis of dichotomous data (gamma, logistic, probit, Weibull, log-logistic, multistage, log-probit, quantal-linear, quantal-quadratic), continuous data (linear, polynomial, power, Hill) and nested developmental toxicology data (NLogistic, NCTR, Rai & Van Ryzin). Results from all models include a reiteration of the model formula and model run options chosen by the user, goodness-of-fit information, a graphical presentation for visual inspection and the concentration estimate for the response at the designated $BMCL_x$, as well as the corresponding BMC_x. More details on the modeling results are described and presented in the analysis on dichotomous data following.

The U.S. EPA benchmark dose (BMD/C) methods guidance has not been finalized at this time to provide definitive procedures and criteria (U.S. EPA 1995). Therefore, in this document provisional criteria for minimum data to perform a benchmark analysis are designated such that (1) complete quantitative information on the response of interest should be available (e.g.,

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[1]For increases in incidence "extra risk" is used which is response incidence (inc) normalized to the background (BG) incidence; response – BG/1-BG.

incidence as number affected / total, means with variability) and that (2) at least two exposure levels with responses that differ from those of the controls are provided, and (3) a benchmark response of 10% is employed such that outcomes are $BMCL_{10}$s. A response of 10% is at or near the limit of sensitivity in most long-term bioassays as determined from both the typical number of animals used in bioassays and a low spontaneous background rate (e.g., 0.1%) for a given effect (Haseman, 1984; Haseman et al., 1989).

B-2. DIESEL DATA FOR BENCHMARK ANALYSIS

Using the criteria set forth in Section B-1 and the information about the critical effects that have been identified (pulmonary inflammation, pulmonary histopathology including indicators of fibrotic changes such as increases in alveolar-capillary wall thickness) the following rat chronic studies identified in Chapter 6 were analyzed for information suitable for BMC analysis: Ishinishi et al. (1986, 1988), Mauderly et al. (1987a,b; 1988); Heinrich et al. (1986, 1995), and Nikula et al. (1995).

Results from this analysis yielded only a few data sets from a single study, that of Nikula et al. (1995), that could be used for BMC analysis. The basis for not including data from the other studies varied. Information on pulmonary histopathology in the studies of Ishinishi et al. (1986, 1988), for example, was supplied only in narrative form with no quantitative information given. A similar situation was found for those reports of the ITRI study; Wolff et al. (1987) reports on clearance alterations due to DPM exposure; Henderson et al. (1988) does give information on hydroxyproline but only in graphical form; the 1988 study of Mauderly et al. deals with pulmonary function as a function of DPM lung loading; the 1987a reference of Mauderly et al. discusses tumor prevalence only and the Mauderly 1987b reference reports on diesel exhaust in developing lung to a single exposure concentration of DPM with no dose-response information available. Those reports on the General Motor study contain extensive information relating not to the critical effects, but mostly to precursors of inflammation such as levels of polymorphonuclear neutrophils and lymphocytes in bronchoalveolar lavage from DPM exposed rats (Strom, 1984) and guinea pigs (Barnhart et al., 1981) as well as information on collagen biosynthesis (Misiorowski et al., 1980) all of which is presented in graphical rather than tabular form amenable for benchmark analysis. The information on noncancer histopathology reported by Heinrich et al. (1995) is in text form only and this author's 1986 study deals primarily with clearance and mortality. Nikula et al. (1995), however, do present extensive quantitative dose-response information (incidence / dichotomous data) on several measures of the critical effect including chronic inflamation (presence of focal aggregates of neutrophils), focal fibrosis with epithelial hyperplasia (nodular fibrosis rimmed by hyperplasia), and septal fibrosis (interstitial fibrosis within alveolar septa) although the study had but 2 exposure

concentrations both of which are different from the controls, a minimal number on which benchmark analysis should be performed.

B-3. BENCHMARK ANALYSIS OF DIESEL DATA

These data from Nikula et al. (1995) were extracted, HEC concentrations calculated using the model of Yu et al. (1991; Appendix A), and analyzed using all 9 applicable models for dichotomous data. Because the benchmark models were ran with the HEC, general from the model of Yu et al. (1991), the $BMCL_{10s}$ are also HECs. The results and data are presented in Table B-1. Results were evaluated based on the nature of the data set, visual inspection of the graphical output, and on the goodness-of-fit parameters, including p values and the AIC. When p values were generated for model fits, values for p that were less than 0.1 were considered to reflect a minimal fit to the data and were disqualified from further consideration. However, the small set of only 3 data points was often matched by the number of parameters fitted in several of the models such that the outcome of the model exactly fit the data and thus no p value is generated; these model fits are often referred to as being overparameterized, and are indicated as "NA" in Table B-1. Values for p that were less than 0.1 were considered to reflect a minimal fit to the data. The AIC (Akaike Information Coefficient; Akaike, 1973; Stone, 1998) is a parameter generated for the models in U.S. EPA (2000) that allows for a general comparison among models run on the same data set. The AIC is defined as -2 log L + 2 p where log L is the log likelihood of the fitted model, and p is the number of parameters estimated; smaller values indicate better fits.

The overall results of this mathematical analysis is reasonable in a biologically mechanistic sense in that chronic inflammation is more prevalent and apparently occurs at lower concentrations (i.e., has lower $BMCL_{10}$ values) than does focal fibrosis. The information on septal fibrosis were not interpretable as the data were not amenable (no or zero background and then total incidence) to any meaningful benchmark or other dose-response analysis. The most sensitive endpoint, chronic inflammation, is therefore the most sensitive benchmark concentration followed by focal fibrosis.

The choice for the most appropriate $BMCL_{10}$ from among the various modeled values for chronic inflammation requires analysis of both the statistical and graphical outputs of the data. The shape of the dose-response curve from information given in Chapter 6 (Table 6-2) gives evidence of considerable "S" character, e.g., several low HECs without any reported effects up to about 0.2 mg/m^3. The shape of the dose-response curves generated by several of the models, including gamma-hit, Weibull, multistage, and quantal linear were all a uniformly upward sloping arc from the origin (graphs not shown) with minimal evidence of any "S" character, a shape not concordant with the data array in Table 6.2. Models that did generate curves with "S"

character included log-logistic, logistic, probit, quantal-quadratic, and log-probit. Because of their concordance with this independent data array on dose-response, the latter outputs are further analyzes.

The results for both chronic inflammation and focal fibrosis for those models with outputs having appreciable "S" character suggest that females may be more sensitive than males for these endpoints as the incidences are higher and the $BMCL_{10}$ values are generally lower for females than for males. However, the model fits of the $BMCL_{10}$s to the chronic inflammation data segregated by sex were generally inadequate as judged from the p values (most being far less than 0.1) or from visual inspection of the fits to the data, several of which (e.g., log-logistic and log-probit) were lacking any appreciable "S" character. However, combining female and male data improved data fitting as judged by the increased p values to where nearly all were >0.1 and to where the visual fits were concordant with the independent information on dose-response. Too, most of the combined $BMCL_{10}$s were either intermediate between the female and male values or somewhat closer to the female values such that the combined $BMCL_{10}$ values were not much different from the females $BMCL_{10}$s.

From among the combined male and female model outputs in Table B-1, the logistic, probit, and quantal quadratic results were all excluded based on the high AIC value relative to the log-logistic and log-probit results. The log-logistic results were excluded based on the shape of the lower portion of the dose-response curve which was upward sloping near the origin (graph not shown) and not as concordant with the independent dose-response information in Table 6-2 as was the fit of the log-probit model (Figure B-1). This leaves the fit of the log-probit model as being most reflective of the information in Table 6-2. The $BMCL_{10}$ of the log-probit curve at 0.37 mg/m^3 remains and, by elimination, appears to be the most defensible choice from among the $BMCL_{10}$s arrayed in Table B-1. Figure B-1 shows the graphical representation of the log-probit model fit to the data and the origin of the $BMCL_{10}$. This graph also shows the relationship of the $BMCL_{10}$ of 0.37 mg/m^3 to the variability that exists around the control value and that the value of 0.37 mg/m^3 is not far removed from the outer range of this variability. The log-probit $BMCL_{10}$ for focal fibrosis (combined) of 1.3 mg/m^3 noted as being representative of this lesion from the BMC analysis in Table B-1.

Characterization of this benchmark value indicates that it may not be a suitable candidate for use as a point of departure for development of a dose-response assessment such as the RfC. An attribute of the benchmark method is that the response (such as the 10% as used here) is near the range of the actual experimental values, such that extrapolation is not far below the observed experimental range. However, due to the paucity of data points overall and lack of any values below an HEC of nearly 2 mg/m^3 in the Nikula et al. (1995) study, the extrapolation of this BMC to the 10% response level is considerable, the $BMLC_{10}$ of 0.37 mg/m^3 being > 5-fold below the

nearest observed value of 1.95 mg/m^3. Also, the high experimental exposures used in this study are in the range of those resulting in pulmonary overload conditions in rats and therefore in the range of the model assumptions of Yu et al. (1991) about this phenomenon in humans for calculation of the HECs (Chapter 3). The $BMCL_{10}$ of 0.37 mg/m^3 is considerably greater than other NOAELs in the DPM data base of 0.144 mg/m^3 and 0.128 mg/m^3 (Table 6-2 in Chapter 6), possibly indicating that these NOAELs represent actual incidence levels that are considerably less than10%; from the same log-probit model the corresponding $BMCL_{05}$ was 0.21 mg/m^3 (near the range of these NOAELs) and the corresponding $BMCL_{01}$ was 0.07 mg/m^3 (below the range of these NOAELs). These limitations on this $BMCL_{10}$ make it a less than optimal candidate for consideration as a point of departure in the development of dose-response assessments.

B-4. SUMMARY

The recently developed EPA Benchmark dose software (U.S. EPA, 2000) and preliminary guidance was utilized to analyze diesel data by the benchmark approach. Data from only one of the array of principal studies identified elsewhere (Chapter 6) was found to contain data amenable to benchmark analysis. The data from this study, that of Nikula et al. (1995) on pulmonary inflammation and histopathology, was extracted and analyzed as dichotomous data using all available models and designating a 10% response level such that $BMCL_{10}$s were calculated; as the models were ran with HECs, the $BMCL_{10s}$ were also HECs.

The analysis resulted in an array of $BMCL_{10}$s from 3 different effects in two sexes (both separate and combined) with 9 different models. These $BMCL_{10}$s were each considered from a perspective of biological relevance, known dose-response character, and from the individual fit to the data by the models from statistical parameters and visual judgments. The $BMCL_{10}$ that emerged after the above considerations was 0.37 mg/m^3 for the combined male plus female incidence of chronic active pulmonary inflammation. A $BMCL_{10}$ of 1.3 mg/m^3 for pulmonary focal fibrosis was also noted in this analysis. Characterization of these benchmark values indicates that neither may be a suitable candidate for use as a point of departure in development of a dose-response assessment such as the RfC but that they are concordant with other quantitative dose-response aspects of the DPM database.

Table B-1. BMC analysis of pathology incidence data in male and female F344 rats from the study of Nikula et al. (1995) using the different models available from U. S. EPA benchmark dose project (U.S. EPA, 2000) for dichotomous data based on 10% extra risk (i.e., a 10% increase relative to a total that has been adjusted for background) and no threshold term. The concentrations used in the analysis are human continuous equivalent concentrations (HECs) obtained from the interspecies extrapolation model of Yu et al. (1991). The table listings include the $BMCL_{10}$ (the benchmark response level of 10% obtained from the lower 95% limit of the benchmark curve in mg/m^3), the BMC_{10} (the corresponding estimate at 10% response from the best fit benchmark curve, also in mg/m^3), P = goodness-of-fit values. NA indicates a G-O-F value was not available, usually due to the lack of degrees of freedom. AIC = Akaike Information Coefficient (see U.S. EPA, 2000 and below) which may be used for model comparison on the same data set.

Effect (from Table 5 and 6, p 86, Nikula et al., 1995)	Inc @ 0 mg/m^3	Inc @ 1.95 mg/m^3 HEC	Inc @ 5.1 mg/m^3 HEC	$BMCL_{10}$ (BMC_{10}) log-logistic	$BMCL_{10}$ (BMC_{10}) log-probit	$BMCL_{10}$ (BMC_{10}) multi-stage	$BMCL_{10}$ (BMC_{10}) - Weibull	$BMCL_{10}$ (BMC_{10}) - gamma	$BMCL_{10}$ (BMC_{10}) - quantal linear	$BMCL_{10}$ (BMC_{10}) - probit	$BMCL_{10}$ (BMC_{10}) - logistic	$BMCL_{10}$ (BMC_{10}) quantal quadratic
Chronic active inflammation >18 mos, grades 1-3, male + female combined	5/177	59/162	118/174	0.32(0.64) P= NA AIC= 483	0.37(.70) P=NA AIC = 483	0.43(.49) P= 0.982 AIC= 481	0.43(.49) P= 0.982 AIC= 481	0.43(.49) P=0.98 AIC= 480	0.43(.49) P= .982 AIC= 481	1.06(1.19) P= 0.000 AIC= 499	1.12(1.26) P=0.000 AIC= 502	1.34(1.45) P= 0.000 AIC = 505
Chronic active inflammation >18 mos, grades 1-3 in males	1/86	19/81	54/85	0.67(1.16) P= NA AIC= 217	0.74(1.22) P = NA AIC = 217	0.56(.95) undefined AIC= 217	.56(1.04) P= NA AIC= 216	.56(1.09) P= NA AIC= 217	0.50(.61) P= 0.15 AIC= 216	1.31(1.55) P= 0.05 AIC= 219	0.67(1.16) P= NA AIC= 217	1.42(1.57) P= 0.055 AIC = 218
Chronic active inflammation >18 mos, grades 1-3 in females	4/91	40/81	64/89	0.18(0.26) P= NA AIC= 257	.016(.30) P = NA AIC = 257	0.33(.40) P= 0.173 AIC= 257	0.33(.40) P= 0.173 AIC= 257	0.33(.40) P= 0.17 AIC= 257	0.33(.40) P= 0.173 AIC= 257	0.83(.96) P= 0.0001 AIC= 272	0.85(1.0) P= 0.000 AIC= 273	1.21(1.35) P= 0.000 AIC = 279
Focal fibrosis with epithelial hyperplasia, grades 1-4 in males and females combined	0/177	18/162	63/174	1.25(1.8) P= 1.000 AIC= 345	1.3(1.8) P = 1.000 AIC = 345	1.21(1.8) P= 1.000 AIC= 345	1.21(1.8) P= 1.000 AIC= 345	1.21(1.8) P= 1.0 AIC= 345	1.1(1.3) P= 0.363 AIC= 345	2.32(2.61) P= 0.013 AIC= 353	2.50(2.8) P= 0.006 AIC= 356	2.14(2.34) P= 0.091 AIC = 347
Focal fibrosis with epithelial hyperplasia, grades 1-4 in males	0/86	5/81	19/85	1.72(2.7) P= 1.00 AIC= 132	1.6(2.7) P = 1.000 AIC = 132	1.79(2.8) undefined AIC= 134	1.79(2.8) P= 1.00 AIC= 132	1.79(2.75 P= 1.0 AIC= 132	1.7(2.4) P= 0.70 AIC= 131	2.98(3.5) P= 0.199 AIC= 134	3.17(3.69) P= 0.153 AIC= 135	2.68(3.1) P=0.552 AIC = 131
Focal fibrosis with epithelial hyperplasia, grades 1-4 in females	0/91	13/81	44/89	0.80(1.4) P= 1.00 AIC= 199	0.87(1.47) P = 1.000 AIC = 199	0.77 P= 0.99 AIC= 199	0.77(1.4) P=1.0 AIC=199	0.71(1.4) P= 1.00 AIC= 199	0.71(.88) P= 0.445 AIC= 198	1.76 P= 0.037 AIC= 205	1.89(2.2) P= 0.02 AIC= 207	1.7(1.9) P= 0.21 AIC = 200
Septal fibrosis, >18 mos, grades 1-4 in males	1/86	79/81	83/85	.003(.008) P= 0.35 AIC= 53	(failed)	0.07(.08) P= 0.000 AIC= 65	0.07(.08) P= 0.000 AIC= 65	0.07(.08) P= 0.000 AIC= 65	0.07(.08) P= 0.000 AIC= 65	0.29(.37) P= 0.000 AIC= 114	0.32(.44) P= 0.000 AIC= 86	0.42(0.47) P 0.000 AIC = 100
Septal fibrosis, >18 mos, grades 1- 4 in females	2/91	75/81	87/89	0.009 (.05) P= NA AIC= 87	(failed)	0.08(.10) P= 0.003 AIC = 91	0.08(.10) P= 0.000 AIC= 91	0.08(.10) P= 0.003 AIC= 91	0.08(.10) P= 0.003 AIC= 91	0.32(.40) P 0.000 AIC= 131	0.34(.45) P= 0.000 AIC= 109	0.46(.51) P 0.000 AIC = 119

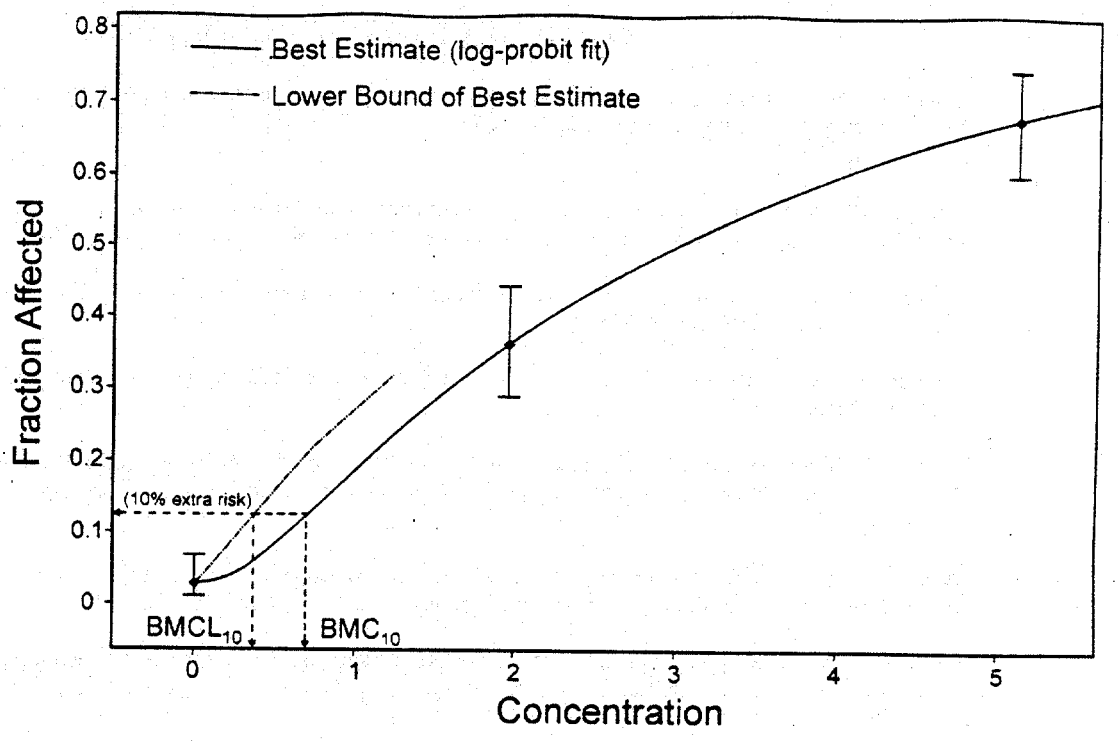

Figure B-1. Benchmark concentration analysis (log-probit) of chronic pulmonary inflammation in rats exposed to DPM from Nikula et al. (1995). $BMCL_{10}$, the lower confidence estimate of the concentration of DPM associated with a 10% incidence (extra risk); BMC_{10}, the corresponding estimate from the best (log-probit) fit. (◊) data with 95% error bounds.

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Appendix C

A Summary Review of Cancer Dose-response Analyses on Diesel Exhaust

C.1. INTRODUCTION

Several individuals and organizations have previously conducted dose-response assessments to estimate quantitatively the cancer risk from exposures to DE. Estimations were performed on the basis of either epidemiologic and/or experimental data. As concluded in Section 8.5, EPA finds that available epidemiologic data are too uncertain to confidently derive a unit risk estimate for DE-induced lung cancer, and that rat data are not suitable for estimating human risk. Nevertheless, a review of historical dose-response evaluations is provided here as background information. This information is not intended to constitute endorsement or a recommendation for use in quantitative risk assessment.

Early analyses to quantitatively assess the carcinogenicity of DE were hindered by a lack of positive epidemiologic studies and long-term animal studies. One means of overcoming these obstacles was the use of comparative potency methods based on combined epidemiologic and experimental data. By the late 1980s, the availability of dose-response data from animal bioassays and epidemiologic studies provided an opportunity for the derivation of both animal and human data-based estimates, although considerable uncertainties were generally acknowledged by the authors of these assessments.

C.2. COMPARATIVE POTENCY METHODS

In this method, the potency of diesel particulate matter (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 extract to each of these agents is then multiplied by their individual unit risk estimates to obtain the unit risk for DE. If epidemiology-based estimates from more than one pollutant are used, the derived potencies are generally averaged to obtain an overall mean. Major uncertainties of this method include the assumptions that (1) the cancer potency of DE can be determined on the basis of the relative effectiveness of the organic fraction alone; (2) the relative potency in short-term tests is an accurate predictor of lung cancer potency; and (3) DPM extracts are similar in chemical composition and proportion as combustion or pyrolysis products.

In the study by Albert et al. (1983), epidemiology-based unit cancer risk estimates for coke oven emissions, cigarette smoke condensate, and roofing tar were used. Samples of DPM 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 DPM extracts were tested in a variety of assays. Dose/concentration-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, resulted in strong dose-response data. Because skin tumor initiation was considered to be the most biologically relevant test, it was used to derive potency estimates for the Nissan engine. An estimate for the Nissan engine was then derived by multiplying the epidemiology-based potency estimates for each of the three agents (coke oven emissions, roofing tar, and cigarette smoke condensate) by the ratios of their potencies in the skin

tumor initiation test to that of the Nissan diesel engine. According to this method, three 95% upper-bound estimates of lifetime cancer risk per microgram per cubic meter of extractable organic matter were derived for the Nissan diesel, based on potency comparisons with each of the three agents. These values are: coke oven emissions, 2.6×10^{-4}; roofing tar, 5.2×10^{-4}; and cigarette smoke condensate, 5.4×10^{-4}. The average of the three equals 4.4×10^{-4}.

The potency of the other diesel emission samples was not estimated directly because of the weak response in the skin tumor initiation test. Instead, their potency relative to the Nissan engine was estimated as the arithmetic mean of their potency relative to the Nissan in the Salmonella assay in strain TA98, the sister chromatid exchange assay in Chinese hamster ovary cells, and the mutation assay in mouse lymphoma cells. The estimated lifetime cancer risk per microgram per cubic meter of extractable organic matter for extracts from these engines are as follows: Volkswagen, 1.3×10^{-4}; Oldsmobile, 1.2×10^{-4}; and Caterpillar, 6.6×10^{-6}.

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 DE cancer potency estimates 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 DPM extract in each of the three bioassays. Harris (1983) derived an overall

mean relative risk value of 3.5×10^{-5} per $\mu g/m^3$ 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.

McClellan (1986), Cuddihy et al. (1981, 1984), and Cuddihy and McClellan (1983) estimated a risk of about 7.0×10^{-5} per $\mu g/m^3$ 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).

C.3. EPIDEMIOLOGY-BASED ESTIMATION OF CANCER RISK

The first lung cancer risk estimates based on epidemiologic data were derived by Harris (1983). He assessed the risk of exposure to DE 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 groups, 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 that 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 5×10^{-4} per $\mu g/m^3$ DPM per year.

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. The number of excess cancer deaths per year in the United States was estimated to be 3,800 (95% C.I. 400-7400 when an exposure of 125 $\mu g/m^3$ was used, and 950 (95% C.I. 100-1,900) when 500 $\mu g/m^3$ DPM was used.

The California EPA (Cal-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 in exposure from 1959 to 1980. The lowest lifetime risk estimate derived was 1.3×10^{-4} per $\mu g/m^3$ and the highest was 2.4×10^{-3} per $\mu g/m^3$. The geometric mean was 6×10^{-4} per $\mu g/m^3$.

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 1990 exposure measurements for different job categories and then retrospectively estimating from 1982 to about 1950 using various factors, including diesel vehicle miles traveled and engine emission rates per mile. The 1990 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 g/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%). Using the same data base, Stayner et al (1998) presented an estimate of excess lifetime risk of 4.5E-4 for a worker exposed to 1 $\mu g/m^3$ of DE for 45 years.

C.4. 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. A high degree of uncertainty exists in the use of the rat data to predict human risk. Major uncertainties include: (1) differences in particle deposition patterns between rats and humans, (2) differences in sensitivity between rats and humans to the carcinogenic action of DE, and (3) extrapolation of rat lung tumor responses

at high concentrations to ambient concentrations without a clear understanding of the mode of action of DE. It is now widely recognized that the rat lung tumor response associated with any insoluble particles at high concentrations is mediated by a particle-overload mechanism (ILSI, 2000), suggesting that rat data for DE are not suitable for estimating human risk at low environmental concentrations.

The first risk estimate was reported by Albert and Chen (1986), 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) also used a linear model and data reported by Brightwell et al. (1989), Heinrich et al. (1986), and Mauderly et al. (1987). They reported risk estimates ranging from 6×10^{-5} to 12×10^{-5} per $\mu g/m^3$. Smith and Stayner (1990), using time-to-tumor models based on the data of Mauderly et al. (1987), derived point (MLE) estimates ranging from 1.0×10^{-4} to 2.1×10^{-4} per $\mu g/m^3$ after converting from occupational to environmental exposure scenario.

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 1.4×10^{-5} per $\mu g/m^3$ when dose was based on carbon particulate matter per unit lung surface area rather than whole DPM, and 1.2×10^{-4} per $\mu g/m^3$ when based on lung burden per unit body weight.

Hattis and Silver (1994) derived a maximum likelihood estimate for occupational exposure of 5.2×10^{-5} per $\mu g/m^3$ 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. California EPA (CAL-EPA, 1998) derived a geometric mean estimate of 6×10^{-5} per $\mu g/m^3$ from five bioassays using an LMS model.

To demonstrate the possible influence of particle effects as well as particle-associated organics, an additional modeling approach was conducted by Chen and Oberdorster (1996). Employing a biologically based two-stage model and using malignant tumor data from Mauderly et al. (1987), 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 DE-induced lung tumor at high exposure concentrations may differ from that at low exposure concentrations, with the organics and particles playing primary roles of tumorigenesis, respectively, at low and high concentrations. Overall, the potency estimates on the basis of

animal bioassays are in the range of 10^{-6} to 10^{-4} per 1 $\mu g/m^3$ of DPM.

Valberg and Crouch (1999) conducted a meta-analysis of rat bioassays by pooling together data of low-dose groups from different bioassays. There are eight bioassays used in the meta-analysis; half of them had duration of 24 months, and the remaining studies had duration of 30 months or more. Animals with continuous lifetime exposure of less than 600 $\mu g/m^3$ of DE were included in the analysis. Continuous lifetime exposure is calculated by protracting actual DE exposure to 30 months (24 hours per day, 7 days per week). The researchers concluded that exposure of rats to DE at concentrations not associated with lung overload is consistent with no tumorigenic effect.

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Exhibit E

1: Am J Ind Med 1998 Sep;34(3):220-8

Diesel exhaust and lung cancer in the trucking industry: exposure-response analyses and risk assessment.

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BACKGROUND: Diesel exhaust is considered a probable human carcinogen by the International Agency for Research on Cancer (IARC). The epidemiologic evidence rests on studies of lung cancer among truck drivers, bus drivers, shipyard workers, and railroad workers. The general public is exposed to diesel exhaust in ambient air. Two regulatory agencies are now considering regulating levels of diesel exhaust: the California EPA (ambient levels) and the Mine Safety Health Administration (MSHA) (occupational levels). To date, there have been few quantitative exposure-response analyses of diesel and lung cancer based on human data. METHODS: We conducted exposure-response analyses among workers in the trucking industry, adjusted for smoking. Diesel exhaust exposure was estimated based on a 1990 industrial hygiene survey. 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 trucks' exhaust systems for long-haul drivers. RESULTS: Regardless of assumptions about past exposure, all analyses resulted in significant positive trends in lung cancer risk with increasing cumulative exposure. A male truck driver exposed to 5 micrograms/m3 of elemental carbon (a typical exposure in 1990, approximately five times urban background levels) would have a lifetime excess risk of lung cancer of 1-2% above a background risk of 5%. CONCLUSIONS: We found a lifetime excess risk ten times higher than the 1 per 1,000 excess risk allowed by OSHA in setting regulations. There are about 2.8 million truck drivers in the U.S. Our results depend on estimates about unknown past exposures, and should be viewed as exploratory. They conform reasonably well to recent estimates for diesel-exposed railroad workers done by the California EPA, although those results themselves have been disputed.

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