7. CARCINOGENICITY OF DIESEL EXHAUST

7.1. INTRODUCTION

Initial health hazard concerns regarding the potential carcinogenicity of diesel exhaust were based on the reported induction of skin papillomas by diesel particle extracts (Kotin et al., 1955), evidence for mutagenicity of extracts (Huisingh et al., 1978), evidence that components of diesel extract act as weak tumor promoters (Zamora et al., 1983), and the knowledge that diesel particles and their associated organics are respirable. During the 1980s, both human 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 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 diesel exhaust particles.

7.1.1. Overview

This chapter evaluates the carcinogenic potential of diesel exhaust in both humans (Section 7.2) and animals (Section 7.3), determines likely mode/s of action (Section 7.4), and provides an overall weight of evidence (Section 7.5) for carcinogenicity in humans. This assessment focuses on diesel exhaust, although diesel particles comprise a portion of ambient particulate matter (PM). In 1998, diesel emissions constituted 72% (521,000 tons) of mobile sources PM_{10} and 18% of total PM_{10} in ambient air (excluding natural and miscellaneous emissions). Diesel emissions made up 77% (473,000 tons) of mobile source $PM_{2.5}$ emissions, and 23% of total $PM_{2.5}$ in ambient air (excluding natural and miscellaneous emissions) in 1998. Ambient PM, notably PM_{10}, has been known for many years to potentially affect human health; these effects have been evaluated in a separate document (U.S. EPA, 1996a). This document is also undergoing revision.

7.1.2. Ambient PM-Lung Cancer Relationships

A quick overview of the data regarding exposure to ambient PM and lung cancer is provided as background information. With DE being part of ambient PM, the question of what is

seen in the ambient PM data is of interest, since insight about the ambient PM exposure lung cancer relationships may contribute to evaluation of DE-specific epidemiologic data.

Chapter 5 noted that (a) DPM, consisting mostly of fine particles (<1.0 μm diameter), represents a toxicologically important component of typical ambient fine particle mixes, and (b) health risk estimates for ambient fine particles would, logically, likely represent an upper limit for estimates of the health risks associated with exposures to DPM as a subset of ambient fine PM. Chapter 5 (and Appendix C) went on to summarize key epidemiologic findings from studies of ambient PM noncancer effects, which provided important inputs to the setting, in 1997, of new ambient fine particle standards ($PM_{2.5}$ NAAQS) to protect against mortality and morbidity effects of airborne fine particles. Several large-scale prospective studies (Harvard Six City Study; American Cancer Society or ACS Study; Adventist Health Study of Smog or AHSMOG) were highlighted in Chapter 5 and Appendix C as providing 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). The same studies also evaluated relationships between chronic PM exposures and lung cancer mortality and/or incidence, evaluations of much pertinence here to consideration of ambient PM cancer risks as possibly representing upper limits for DPM-related cancer risks.

The Harvard Six City Study (Dockery et al., 1993), of approximately 8,000 adults in six cities comprising a transect across the northcentral and northeastern United States, found markedly increased relative risks (RR) of lung cancer mortality for current (RR = 8.00, 95% CL 2.97-21.6) and former (RR = 2.54, CL 0.90-7.18) smokers. Also, elevated but statistically nonsignificant associations of lung cancer mortality risks (RR = 1.37, CL 0.81-2.31) were found by the Six City Study analyses (which included data for both males and females) to be related to ambient fine particles indexed by a range of annual mean $PM_{2.5}$ concentrations from the least to the most polluted of the six cities.

The ACS Study (Pope et al., 1995), of 550,000 adults in 151 cities across all U.S. geographic regions, also found markedly elevated lung cancer risks for current smokers (RR = 9.73, CL 5.96-15.9) and somewhat elevated and statistically significant lung cancer risk (RR = 1.36, CL 1.11-1.66) associations with a range (19.9 mg/m^3) of annual average sulfate (SO_4) concentrations as one index of chronic exposures to ambient fine particles, 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, CL 1.14-1.83) were statistically significant in relation to sulfate concentrations as the fine particle indicator in the 151 cities. Note that the analogous adjusted risk ratios (and 95% CL) for the most polluted versus least polluted cities in terms of sulfate levels were statistically

nonsignificant for male "never-smokers" (RR = 1.36, CL 0.40 - 4.66), for female "ever-smokers" (RR = 1.10, CL 0.72-1.68) and female "never-smokers" (RR = 1.61; CL 0.66 -3.92). Also, lung cancer mortality risks (RR = 1.03; CL 0.80-1.33) were not statistically significantly associated with ambient $PM_{2.5}$ concentrations (across a range of 24.5 $\mu g/m^3$ from least to most polluted of a subset of 50 of the 151 cities) in overall combined analyses of data for both males and females. Nor were the relative risk ratios statistically significant for smaller sample size subgroups broken out by sex and smoking status in relation to $PM_{2.5}$ concentrations, as a second index of ambient airborne fine PM. Hence, the ACS Study provides only very limited evidence hinting at a possible lung cancer mortality association with one indicator (sulfates) of ambient fine particles, but not with another such index ($PM_{2.5}$).

In the first of an ongoing series of reports on AHSMOG data analyses, Abbey et al. (1991) described the results of initial analyses related to the AHSMOG evaluation of air pollution effects on the health of 6,338 nonsmoking, long-term California adult residents. Of a variety of health endpoints evaluated, only respiratory symptoms and female cancers (any site) but not respiratory cancer for either sex, were reported by Abbey et al. (1991) to be associated with concentrations of total suspended particulate (TSP) matter (which includes not only fine particles indexed by $PM_{2.5}$ but also larger coarse mode particles ranging up to 25-50 mm). Later follow-up analyses (Abbey et al., 1995) considered chronic exposures to PM_{10} (estimated from TSP data), $PM_{2.5}$ (estimated from visibility data), and SO_4, but found no statistically significant associations with nonexternal mortality. Subsequent AHSMOG analyses reported out by Abbey et al. (1999) and Beesan et al. (1998) do, however, hint at possible associations of increased risk of lung cancer mortality and/or incidence in males with ambient PM exposures. More specifically, chronic exposures to ambient PM_{10} (which includes both fine particle and <2.5 mm and coarse particles 2.5 to 10 mm in size) were reported to be significantly associated with markedly increased lung cancer mortality risks in the nonsmoking AHSMOG males (RR = 23.39, CL 2.55-60.10), but not for the females (RR = 9.8; CL 0.34-9.52). Male lung cancer mortality was also reported to be significantly associated with numbers of days per year that PM_{10} exceeded 100 mg/m^3 (RR = 1.055, CL 0.66-1.69). Other analyses of AHSMOG data were reported by Beeson et al. (1998) also showing statistically significant associations of increased lung cancer incidence (especially $PM_{10} > 100$ $\mu g/m^3$) for males, but not for females.

In summary, the three key prospective cohort studies (discussed in more detail in Appendix C) provide an equivocal array of results with regard to possible associations between chronic exposures to ambient PM and lung cancer mortality and/or incidence. Only the ACS Study found a statistically significant association of increased risk of lung cancer with one indicator of ambient fine particles (sulfates), but not with another such indicator ($PM_{2.5}$)–the latter

being consistent with Harvard Six City Study results for $PM_{2.5}$. Also, the AHSMOG results hint at possible increased lung cancer risks in males, but not females, in relation to PM_{10} levels. Overall, then, these studies are not conclusive and appear, at best, only to provide some indication of possible associations between increased lung cancer risk and chronic ambient fine PM exposures.

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 diesel emissions. Isolated authors have reported other malignancies, including testicular cancer (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 lung cancer studies is presented in this section. 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 diesel exhaust 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.

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. 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 diesel exhaust 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 diesel exhaust 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 diesel exhaust. 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 diesel exhaust (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.

7.2.1. Cohort Studies

7.2.1.1. *Waller (1981): Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes*

A retrospective mortality study of a cohort of London transport workers was conducted to determine if there was an excess of deaths from lung cancer that could be attributed to diesel exhaust exposure. 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 concentrations and was compared with corresponding values recorded in 1957. Concentrations of benzo[*a*]pyrene recorded in 1957 were at least 10 times greater than those measured in 1979.

This study failed to find any association between diesel exhaust 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 diesel exhaust, 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 job categories either by qualitative or quantitative levels of diesel exhaust 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 benzo[*a*]pyrene concentrations suggests that the cohort in its earlier years was exposed to much higher concentrations of environmental contaminants than currently exist. It is not clear when the reduction in benzo[a]pyrene concentration occurred, because there are no environmental readings available between 1957 and 1979. It is also important to note that the concentrations of benzo[*a*]pyrene inside the garage in 1957 were not very different from those outside the garage, thus indicating that exposure for garage workers was not much different from that of the general

population. Thus, this study fails to provide any negative association between the diesel exhaust 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 $\geq$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 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 diesel exhaust, respectively, with a highly significant trend ($p<0.001$).

An analysis done on individuals who retired prior to 1950 showed the relative risk of lung cancer among nonexposed, possibly exposed, and probably exposed to be 1.00, 0.70, and 0.44, respectively, based on fewer than 15 deaths in each category. A similar analysis of individuals who retired after 1950 found the results in the same categories to be 1.00, 1.23, and 1.40, respectively. Although retirement prior to 1950 indicated exposure to coal 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 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 diesel exhaust versus those not exposed. Despite these limitations, this study provides suggestive evidence toward a causal association between exposure to diesel exhaust 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 diesel exhaust exposure, however. The reason for leaving was coded as died in service, retired, or other. The underlying cause of death was coded using the eighth revision of the International Classification of Diseases (ICD). Person-years were calculated from date of birth and dates of entry to and exit from the study using the man-years computer language program. 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.

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 diesel exhaust 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 Diesel Exhaust 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 diesel exhaust emissions. Study cohort members were identified from records maintained at Operating Engineers' Local Union No. 3-3A in San Francisco, CA. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 year of membership in this union between January 1, 1964, and December 31, 1978, were included in the study. Work histories of the cohort were obtained from job dispatch computer tapes. The study follow-up period was January 1964 to December 1978. Death information was obtained from a trust fund, which provided information on retirement dates, vital status, and date of death for those who were entitled to retirement and death benefits. Approximately 50% of the cohort had been union members for less than 15 years, whereas the other 50% had been union members for 15 years or more. The average duration of membership was 15 years. As of December 31, 1978, 29,046 (85%) cohort members were alive, 3,345 (9.8%) were dead, and 1,765 (5.2%) remained untraced. Vital status of 10,505 members who had left the union as of December 31, 1978, was ascertained from the Social Security Administration. Death certificates were obtained from appropriate State health departments. Altogether, 3,243 deaths (for whom death certificates were available) in the cohort were coded using the seventh revision of the ICD. For 102 individuals, death certificates could not be obtained, only the date of death; these individuals were included in the calculation of the SMR for all causes of death but were deleted from the cause-specific SMR analyses. Expected deaths and SMRs were calculated using the U.S. national age-sex-race cause-specific mortality rates for 5-year time periods between 1964 and 1978. The entire cohort population contributed to 372,525.6 person-years in this 5-year study period.

A total of 3,345 deaths was observed, compared with 4,109 expected. The corresponding SMR for all causes was 81 (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 diesel exhaust 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 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 who had the same job before and after 1967 and thus may have worked 12 to 14 years or longer. If they had belonged to the category in which heavy exposure to diesel exhaust emissions was very common for this prolonged time, then the increase in lung cancer, as well as stomach cancer, might be linked to diesel exhaust. Further information on those without work histories should be obtained if possible, because such information may be quite informative with regard to the evaluation of the carcinogenicity of diesel exhaust.

The study design is adequate, covers about a 15-year observation period, has a large enough population, and is appropriately analyzed; however, it has too many limitations to permit any conclusions. First, no exposure histories are available; one has to make do with job histories, which provide limited information on exposure level. Any person who ever worked at the job, or any person working at the same job over any period of time, is included in the same category; this would have a dilution effect, 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 Diesel Exhaust***

This retrospective cohort mortality study of bus company employees investigated a possible increased mortality of cardiovascular diseases and cancers from diesel exhaust exposure. The cohort comprised all males employed at five different bus companies in southeastern Sweden between 1950 and 1959. Based on information from personnel registers, individuals were classified into one or more categories and could have contributed person-years at risk in more than one exposure category. The study period was from 1951 to 1983; information was collected from the National Death Registry, and copies of death certificates were obtained from the National Bureau of Statistics. Workers who died after age 79 were excluded from the study because diagnostic procedures were likely to be more uncertain at higher ages (according to investigators). The cause-, sex-, and age-specific national death rates in Sweden were applied to the 5-year age categories of person-years of observation to determine expected deaths for all causes, malignant diseases, and cardiovascular diseases. A Poisson distribution was used to calculate *p*-values and confidence limits for the ratio of observed to expected deaths. The total cohort of 694 men (after loss of 5 men to follow-up) was divided into three exposure categories: (1) clerks with lowest exposure, (2) bus drivers with moderate exposure, and (3) bus garage workers with highest exposure.

The 694 men provided 20,304 person-years of observation, with 195 deaths compared with 237 expected. A deficit in cancer deaths largely accounted for this lower-than-expected mortality in the total cohort. Among subcohorts, no difference between observed and expected deaths for total mortality, total cancers, or cardiovascular causes was observed for clerks (lowest diesel exposure), bus drivers (moderate diesel exposure), and garage workers (high diesel exposure). The risk ratios for all three categories were less than 1 except for cardiovascular diseases among bus drivers, which was 1.1.

When the analysis was restricted to members who had at least a 10-year latency period and either any exposure or an exposure exceeding 10 years, similar results were obtained, with fewer neoplasms than expected, whereas cardiovascular diseases showed risk around or slightly above unity.

Five lung cancer deaths were observed among bus drivers who had moderate diesel exhaust exposure, whereas seven were expected. The only other lung cancer death was observed among bus garage workers who had the highest diesel exhaust exposure. This study's major limitations, including small size and poor data on diesel exhaust exposure, make it inadequate to draw any conclusions.

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

Boffetta and Stellman conducted a mortality analysis of 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 diesel exhaust and to employment in selected occupations related to diesel exhaust 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 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 diesel exhaust exposure, mortality for all causes and lung cancer in relation to

employment in selected occupations with high diesel exhaust exposure, and mortality from other causes in relation to diesel exhaust exposure. The incidence-density ratio was used as a measure of association, and test-based confidence limits were calculated by the Miettinen method. For stratified analysis, the Mantel-Haenszel method was used for testing linear trends. 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 diesel exhaust exposure and were therefore excluded from the main diesel exhaust analysis. Among individuals who had provided diesel exhaust exposure history, 62,800 were exposed and 307,143 were not exposed. Comparison of the population with known information on diesel exhaust exposure with the excluded population with no information on diesel exhaust exposure showed that the mean ages were 54.7 and 57.7 years, the nonsmokers were 72.4% and 73.2%, and the total mortality rates per 1,000 per year were 23.0% and 28.8%, respectively.

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 diesel exhaust exposure was examined. For drivers who worked for 1 to 15 years, the relative risk was 0.87, 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 diesel exhaust exposure showed a significant excess of deaths ($p<0.05$) in the following categories: cerebrovascular disease, arteriosclerosis, pneumonia, influenza, cirrhosis of the liver, and accidents.

The 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 diesel exhaust exposure. None of this information was validated. Nearly 20% of the individuals had an unknown exposure status to diesel exhaust, and they experienced a higher mortality for all causes

and lung cancer than both the diesel exhaust exposed and unexposed groups. This could have introduced a substantial bias in the estimate of the association. Given that all diesel exhaust exposure occupations, such as heavy equipment operators, truck drivers, and railroad workers, showed elevated lung cancer risk, this study is suggestive of a causal association. 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 diesel exhaust exposure. This study did not find any association between exposure to diesel exhaust and bladder cancer.

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

An earlier case-control study of lung cancer and diesel exhaust exposure in U.S. railroad workers by these investigators had demonstrated a relative odds of 1.41 (95% CI = 1.06, 1.88) for lung cancer with 20 years of work in jobs with diesel exhaust exposure. To confirm these results, a large retrospective cohort mortality study was conducted by the same investigators. Data sources for the study were the work records of the U.S. Railroad Retirement Board (RRB). The cohort was selected based on job titles in 1959, which was the year by which 95% of the locomotives in the United States were diesel powered. Diesel exhaust exposure was considered to be a dichotomous variable depending on yearly job codes between 1959 and death or retirement through 1980. Industrial hygiene evaluations and descriptions of job activities were used to classify jobs as exposed or unexposed to diesel emissions. A questionnaire survey of 534 workers at one of the railroads where workers were asked to indicate the amount of time spent in railroad locations, either near or away from sources of diesel exhaust, was used to validate this classification. Workers selected for this survey were actively employed at the time of the survey, 40 to 64 years of age, 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 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 calender 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 diesel exhaust-exposed jobs in 1959 and by cumulative number of years of diesel exhaust exposure through 1980. Directly standardized rate ratios for deaths from lung cancer were calculated for diesel exhaust exposed compared with unexposed for each 5-year age group in 1959. The standardized rates were based on the overall 5-year person-year time distribution of individuals in each age group starting in 1959. The only exception to this was between 1979 and 1980, when a 2-year person-year distribution was used. The Mantel-Haenszel analogue for person-year data was used to calculate 95% confidence intervals for the standardized rate ratios.

The cohort consisted of 55,407 workers, 19,396 of whom had died by the end of 1980. Death certificates were not available for 11.7% of all deaths. Of the 17,120 deaths for whom death certificates were obtained, 48.4% were attributable to diseases of the circulatory system, whereas 21% were attributable to all neoplasms. Of all neoplasms, 8.7% (1,694 deaths) were due to lung cancer. A higher proportion of workers in the younger age groups, mainly brakemen and conductors, were exposed to diesel exhaust, while a higher proportion of workers in the older age groups were potentially exposed to asbestos. In a proportional hazards model, analyses by age in 1959 found a relative risk of 1.45 (95% CI = 1.11, 1.89) among the age group 40 to 44 years and a relative risk of 1.33 (95% CI = 1.03, 1.73) for the age group 45 to 49 years. Risk estimates in the older age groups 50 to 54, 55 to 59, and 60 to 64 years were 1.2, 1.18, and 0.99, respectively, and were not statistically significant. The two youngest age groups in 1959 had workers with the highest prevalence and longest duration of diesel exhaust exposure and lowest exposure to asbestos. When potential asbestos exposure was considered as a confounding variable in a proportional hazards model, the estimates of relative risk for asbestos exposure were all near null value and not significant. Analysis of workers exposed to diesel exhaust in 1959 (n = 42,535), excluding workers with potential past exposure to asbestos, yielded relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) in the 1959 age groups 40 to 44 years and 45 to 49 years. Directly standardized rate ratios were also calculated for each 1959 age group based on

diesel exhaust exposure in 1959. The results confirmed those obtained by using the proportional hazards model.

Relative risk estimates were then obtained using duration of diesel exhaust exposure as a surrogate for dose. In a model that used years of exposure up to and including exposure in the year of death, no exposure duration-response relationship was obtained. When analysis was done by disregarding exposure in the year of death and 4 years prior to death, the risk of dying from lung cancer increased with the number of years worked in a diesel-exhaust-exposed job. In this analysis, exposure to diesel exhaust was analyzed by exposure duration groups and in a model entering age in 1959 as a continuous variable. The workers with greater than 15 years of exposure had a relative risk of lung cancer of 1.72 (95% CI = 1.27, 2.33). The risk for 1 to 4 years of cumulative exposure was 1.20 (95% CI = 1.01, 1.44); for 5 to 9 years of cumulative exposure, it was 1.24 (95% CI = 1.06, 1.44); and for 10 to 14 years of cumulative exposure, it was 1.32 (95% CI = 1.13, 1.56).

The results of this study, demonstrating a positive association between diesel exhaust exposure and increased lung cancer, are consistent with the results of the case-control study conducted by the same investigators in railroad workers dying of lung cancer from March 1981 through February 1982. This cohort study has addressed many of the weaknesses of the other epidemiologic studies. The large sample size (60,000) allowed sufficient power to detect small risks and also permitted the exclusion of workers with potential past exposure to asbestos. The stability of job career paths in the cohort ensured that of the workers 40 to 44 years of age in 1959 classified as diesel exhaust-exposed, 94% of the cases were still in diesel exhaust-exposed jobs 20 years later.

The main limitation of the study is the lack of quantitative data on exposure to diesel exhaust. This is one of the few studies in which industrial hygiene measurements of diesel exhaust were done. These measurements were correlated with job titles to divide the cohort in dichotomous exposure groups of exposed and nonexposed. This may have led to an underestimation of the risk of lung cancer because exposed groups included individuals with low to high exposure. The number of years exposed to diesel exhaust was used as a surrogate for dose. The dose, based on duration of employment, was inaccurate because 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 diesel exhaust 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 diesel exhaust is associated with a modest risk (1.5) of lung cancer.

The data of this study were reanalyzed by Crump et al. (1991), who 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. Garshick conducted some additional analyses (letter from E. Garshick, Harvard Medical School, to Dr. Chao Chen, U.S. EPA, dated August 15, 1991) and reported that the relationship between years of exposure, when adjusted for attained age, and calendar year is flat to negative depending upon which model was used. They 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. Their 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. Crump (1999) reported that the negative dose-response continued to be upheld in his latest analysis. California EPA (CalEPA, 1998) found a positive dose-response by using age at 1959 but allowing for an interaction term of age and calendar year in the model. A detailed discussion of divergent results observed by Crump and CalEPA can be found in Chapter 8.

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

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

random without replacements. Matched analyses were done to calculate odds ratios using conditional logistic regression. The EGRET and Epilog programs were used for these analyses.

Diesel exhaust and asbestos exposure assessments were performed by industrial hygienists based on the intensity of exposure to diesel exhaust and asbestos, specific for workplace, work task, and calendar time period. A diesel exhaust exposure assessment was based on (1) amount of emission (number of buses, engine size, running time, and type of fuel), (2) ventilatory equipment and air volume of the garages, and (3) job types and work practices. Based on detailed historical data and very few actual measurements, relative exposures were estimated (these were not absolute exposure levels). The scale was set to 0 for unexposed and 1 for lowest exposure, with each additional unit increase corresponding to a 50% increase in successive intensity (i.e., 1.5, 2.25, 3.38, and 5.06).

Based on personal sampling of asbestos during 1987, exposures were estimated and time-weighted annual mean exposures were classified on a scale of three degrees (0, 1, and 2). Cumulative exposures for both diesel exhaust and asbestos were calculated by multiplying the level of exposure by the duration of every work period. An exposure index was calculated by adding for every individual contribution from all work periods for both diesel exhaust and asbestos. Four diesel exhaust index classes were created: 0 to 10, 10 to 20, 20 to 30, and >30. The four asbestos index classes were 0 to 20, 20 to 40, 40 to 60, and >60. The cumulative exposure indices were used for the nested case-control study.

Excesses were observed for all cancers and some other site-specific cancers using both comparison populations for the cohort mortality study, but none of them was statistically significant. Based on 17 cases, 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 diesel exhaust 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 diesel exhaust 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 diesel exhaust.

The main strength of this study is the detailed exposure matrices constructed for both diesel exhaust and asbestos exposure, although they were based primarily on job tasks and very few actual measurements. There are a few methodological limitations to this study. The cohort is small and there were only 17 lung cancer deaths; thus the power is low. Exposure or outcome may be misclassified, although any resulting bias in the relative risk estimates is likely to be

toward unity, because exposure classification was done independently of the outcome. Although the analysis by dose indices was done, no latency analysis was performed. 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 diesel exhaust. Overall, this study provides some support to the excess lung cancer results found earlier among populations exposed to diesel exhaust.

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

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

Through automatic record linkage between the 1970 census register (the Central Population Register 1970 to 1980) and the Death Certificate Register (1970 to 1980), the population was followed for cause-specific mortality or emigration up to November 9, 1980. Expected number of deaths among truck drivers was calculated by using the 5-year age group and 5-year time period death rates of the unexposed group and applying them to the person-years accumulated by truck drivers. ICD Revision 8 was used to code the underlying cause of death. Test-based CIs were calculated using Miettinen's method. A Poisson distribution was assumed for the smaller numbers, and CI was calculated based on exact Poisson distribution (Ciba-Geigy). Total person-years accrued by truck drivers were 138,302, whereas for the unexposed population, they were 407,780. There were 627 deaths among truck drivers and 3,811 deaths in the unexposed group. Statistically significant excesses were observed for all cancer mortality (SMR = 121, 95% CI = 104 to 140); cancer of respiratory organs (SMR = 160, 95% CI = 128 to 198), which 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. No excess was observed for bladder cancer.

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

any of these truck drivers or any other laborers had changed jobs after the census of November 9, 1970, thus creating potential misclassification bias in exposure to diesel exhaust. The 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 diesel exhaust 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): Diesel Exhaust 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 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 diesel exhaust 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 diesel exhaust and excess lung cancer.

Table 7-1 summarizes the above cohort studies.

7.2.2. Case-Control Studies of Lung Cancer

7.2.2.1. *Hall and Wynder (1984): A Case-Control Study of Diesel Exhaust Exposure and Lung Cancer*

Hall and Wynder (1984) conducted a case-control study of 502 male lung cancer cases and 502 controls without tobacco-related diseases that examined an association between occupational diesel exhaust exposure and lung cancer. Histologically confirmed primary lung cancer patients who were 20 to 80 years old were ascertained from 18 participating hospitals in 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 diesel exhaust exposure and risk of lung cancer incidence. Summary odds ratios were computed by the Mantel-Haenszel method after adjusting for potential confounding by age, smoking, and socioeconomic class. Two-sided, 95% confidence intervals were computed by Woolf's method. Occupational exposure to diesel exhaust was defined by two criteria. First, occupational titles were coded "probably high exposure" as defined by the industrial hygiene standards established for the various jobs. The job titles included under this category were warehousemen, bus and truck drivers, railroad workers, and heavy equipment operators and repairmen. The second method used the National Institute for Occupational Safety and Health (NIOSH) criteria to analyze occupations by diesel exposure. In this method, the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10% to 19% of their employees exposed were defined as "moderate exposure," and those with less than 10% of their employees exposed were defined as "low exposure."

Cases and controls were compared with respect to exposure. The relative risk was 2.0 (95% CI = 1.2, 3.2) for those workers who were exposed to diesel exhaust versus those who

were not. The risk, however, decreased to a nonsignificant 1.4 when the data were adjusted for smoking. Analysis by NIOSH criteria found a nonsignificant relative risk of 1.7 in the high-exposure group. There were no significantly increased cancer risks by occupation either by the first method or by the NIOSH method. To assess any possible synergism between diesel exhaust exposure and smoking, the lung cancer risks were calculated for different smoking categories. The relative risks were 1.46 among nonsmokers and ex-smokers, 0.82 among current smokers of <20 cigarettes/day, and 1.3 among current smokers of 20+ cigarettes/day, indicating a lack of synergistic effects.

The major strength of this study is the availability of a detailed smoking history for all the study subjects. However, this is offset by lack of diesel exhaust exposure measurements, use of a poor surrogate for exposure, and lack of consideration of latency period. Information was collected on only one major lifetime occupation, and it is likely that those workers who had more than one major job may not have reported the occupation with the heaviest diesel exhaust exposures. Furthermore, the exposure categories based on job titles were broad, and thus would have made a true effect of diesel exhaust 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%).

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 ($\geq$1 year of employment) and underground miners ($\geq$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 diesel exhaust 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, diesel exhaust, or varying degrees of both. An excess risk was detected for underground miners, but it is not known if this was due to diesel emissions from engines or from radon daughters in poorly ventilated mines. Although a high response rate (98%) was obtained by the postal questionnaires, the use of surrogate respondents is known to lead to misclassification errors that can bias the 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

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, diesel exhaust, and formaldehyde—the industries and occupations determined to have exposure were identified, and linking of specific industries and occupations was based on literature review and consultation with local industrial hygienists.

The relative odds were calculated for suspect occupations and industries, classifying individuals as ever employed for at least 1 year in an industry or occupation and defining the reference group as those subjects never employed in that particular industry or occupation. Multiple logistic regression models were used to control simultaneously for age, ethnicity, and smoking status. For occupations with potential diesel exhaust exposure, the analysis showed no excess risks for diesel engine mechanics and auto mechanics. Similarly, when analyzed by exposure to specific agents, the odds ratio (OR) adjusted for age, smoking, and ethnicity was not elevated for diesel exhaust 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 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 diesel exhaust exposed based on reported occupations. The observed absence of an association for exposure to asbestos, a well-established lung carcinogen, may be explained by the misclassification errors in exposure status or by sample size constraints (not enough power). Likewise, the association for diesel exhaust reported by only 7 cases and 17 controls also may have gone undetected because of low power. In conclusion, there is insufficient evidence from this study to confirm or refute an association between lung cancer and diesel exhaust exposure.

7.2.2.4. *Garshick et al. (1987): A Case-Control Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers*

An earlier pilot study of the mortality of railroad workers by the same investigators (Schenker et al., 1984) found a moderately high risk of lung cancer among workers exposed to diesel exhaust compared with those who were not. On the basis of these findings the investigators conducted a case-control study of lung cancer in the same population. The population base for this case-control study was approximately 650,000 active and retired male U.S. railroad workers with 10 years or more of railroad service who were born in 1900 or later. The U.S. Railroad Retirement Board (RRB), which operates the retirement system, is separate from the Social Security System, and to qualify for the retirement or survivor benefits the workers had to acquire 10 years or more of service. Information on deaths that occurred between March 1, 1981, and February 28, 1982, was obtained from the RRB. For 75% of the deceased population, death certificates were obtained from the RRB, and, for the remaining 25%, they were obtained from the appropriate State departments of health. Cause of death was coded according to the eighth revision of the ICD. The cases were selected from deaths with primary lung cancer, which was the underlying cause of death in most cases. Each case was matched to two deceased controls whose dates of birth were within 2.5 years of the date of birth of the case and whose dates of death were within 31 days of the date of death noted in the case. Controls were selected

randomly from workers who did not have cancer noted anywhere on their death certificates and who did not die of suicide or of accidental or unknown causes.

Each subject's work history was determined from a yearly job report filed by his employer with the RRB from 1959 until death or retirement. The year 1959 was chosen as the effective start of diesel exhaust exposure for this study since by this time 95% of the locomotives in the United States were diesel powered. Investigators acknowledge that because the transition to diesel-powered engines took place in the early 1950s, some workers had additional exposure prior to 1959; however, if a worker had died or retired prior to 1959, he was considered unexposed. Exposure to diesel exhaust was considered to be dichotomous for this study, which was assigned based on an industrial hygiene evaluation of jobs and work areas. Selected jobs with and without regular diesel exhaust exposure were identified by a review of job title and duties. Personal exposure was assessed in 39 job categories representative of workers with and without diesel exhaust exposure. Those jobs for which no personal sampling was done were considered exposed or unexposed on the basis of similarities in job activities and work locations and by degree of contact with diesel equipment. Asbestos exposure was categorized on the basis of jobs held in 1959, or on the last job held if the subject retired before 1959. Asbestos exposure in railroads occurred primarily during the steam engine era and was related mostly to the repair of locomotive steam boilers that were insulated with asbestos. Smoking history information was obtained from the next of kin.

Death certificates were obtained for approximately 87% of the 15,059 deaths reported by the RRB, from which 1,374 cases of lung cancer were identified. Fifty-five cases of lung cancer were excluded from the study for either incomplete data (20) or refusal by two States to use information on death certificates to contact the next of kin. Successful matching to at least one control with work histories was achieved for 335 (96%) cases $\leq$64 years of age at death and 921 (95%) cases $\geq$65 years of age at death. In both age groups, 90% of the cases were matched with two controls. There were 2,385 controls in the study; 98% were matched within ± 31 days of the date of death, whereas the remaining 2% were matched within 100 days. Deaths from diseases of the circulatory system predominated among controls. Among the younger workers, approximately 60% had exposure to diesel exhaust, whereas among older workers, only 47% were exposed to diesel exhaust.

Analysis by a regression model, in which years of diesel exhaust exposure were the sum total of the number of years in diesel-exposed jobs, used as a continuous exposure variable, yielded an odds ratio of lung cancer of 1.39 (95% CI = 1.05, 1.83) for >20 years of diesel exhaust exposure in the $\leq$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 $\geq$65 years of age group

were not significant. Increasing years of diesel exhaust exposure, categorized as ≥20 diesel years and 5 to 19 diesel years, with 0 to 4 years as the referent group, showed significantly increased risk in the ≤64 years of age group after adjusting for asbestos exposure and pack-year category of smoking. For individuals who had ≥20 years of diesel exhaust exposure, the odds ratio was 1.64 (95% CI = 1.18, 2.29), whereas among individuals who had 5 to 19 years of diesel exhaust exposure, the odds ratio was 1.02 (95% CI = 0.72, 1.45). In the ≥65 years of age group, only 3% of the workers were exposed to diesel exhaust for more than 20 years. Relative odds for 5 to 19 years and ≥20 years of diesel exposure were less than 1 (p>0.01) after adjusting for smoking and asbestos exposure.

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 diesel exhaust exposure and the three pack-year categories (<50, >50, and missing pack-years) were explored. The cross-product terms were not significant. A model was also tested that excluded recent diesel exhaust exposure occurring within the 5 years before death and gave an odds ratio of 1.43 (95% CI = 1.06, 1.94), adjusted for cigarette smoking and asbestos exposure, for workers with 15 years of cumulative exposure. For workers with 5 to 14 years of cumulative exposure, the OR were not significant.

The many strengths of the study are consideration of confounding factors such as asbestos exposure and smoking; classification of diesel exhaust exposures by job titles and industrial hygiene sampling; exploration of interactions between smoking, asbestos exposure, and diesel exhaust exposure; and good ascertainment (87%) of death certificates from the 15,059 deaths reported by the RRB.

The investigators also recognized and reported the following limitations: overestimation of cigarette consumption by surrogate respondents, which may have exaggerated the contribution of smoking to lung cancer risk, and use of the Interstate Commerce Commission (ICC) job classification as a surrogate for exposure, which may have led to misclassification of diesel exhaust exposure jobs with low intensity and intermittent exposure, such as railroad police and bus drivers, as unexposed. These two limitations would result in underestimation of the lung cancer risk. This source of error could have been avoided if diesel exhaust exposures were categorized by a specific dose range associated with a job title that could have been classified as heavy, medium, low, and zero exposure instead of a dichotomous variable. The use of death certificates to identify cases and controls may have resulted in misclassification. Controls may have had undiagnosed primary lung cancer, and lung cancer cases might have been secondary lesions misdiagnosed as primary lung cancer. However, the investigators quote a third National Cancer Survey report in which the death certificates for lung cancer were coded appropriately in

95% of the cases. Last, as in all previous studies, there is a lack of data on the contribution of unknown occupational or environmental exposures and passive smoking. Furthermore, the lung cancer cases were selected between 1981 and 1982, a total of 22 years latency, which is probably short. In conclusion, this study provides strong evidence that occupational diesel exhaust 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, $\leq$20 years, or >20 years), daily consumption of cigarettes (nonsmokers, <20 cigarettes a day, and $\geq$20 cigarettes a day), and duration of cigarette smoking (nonsmokers, <35 years, and $\geq$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.

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 exposure to known and suspected lung carcinogens. OR 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. OR 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. OR 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 OR 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 diesel exhaust 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 diesel exhaust 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.

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 diesel exhaust exposure. Data on job categories were missing for 12% of the study subjects. A second work history file was also created based on the Teamsters Union pension application that lists occupation, employer, and dates of employment. A three-digit U.S. census code for occupation and industry was assigned to each job for each individual. This Teamsters Union work history file did not have information on whether men drove diesel or gasoline trucks, and the four principal occupations were long-haul drivers, short-haul or city drivers, truck mechanics, and dockworkers. Subjects were assigned the job category in which they had worked the longest.

The case-control analysis was done using unconditional logistic regression. Separate analyses were conducted for work histories from the Teamsters Union pension file and from next-of-kin interviews. Covariate data were obtained from next-of-kin interviews. Analyses were also performed for two time periods: employment after 1959 and employment after 1964. These two cut-off years reflect years of presumed dieselization: 1960 for most trucking companies and 1965 for independent driver and nontrucking firms. Data for analysis could be obtained for 994 cases and 1,085 controls using Teamsters Union work history and for 872 cases and 957 controls using next-of-kin work history. When exposure was considered as a dichotomous variable, for both Teamsters Union and next-of-kin work history, no single job category had an elevated risk. From the next-of-kin data, diesel truck drivers had an odds ratio of 1.42 (95% CI = 0.74, 2.47) and diesel truck mechanics had an odds ratio of 1.35 (95% CI = 0.74, 2.47). OR 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 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 diesel exhaust exposure or smoking and diesel exhaust exposure were observed. All the OR 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 diesel exhaust exposure and excess lung cancer.

7.2.2.8. *Steenland et al. (1998): Diesel Exhaust and Lung Cancer in the Trucking Industry: Exposure-Response Analyses and Risk Assessment*

Steenland et al. (1998) conducted an exposure-response analysis by supplementing the data from their earlier case-control study of lung cancer and truck drivers in the Teamsters Union (Steenland et al., 1990) with exposure estimates based on a 1990 industrial hygiene survey of elemental carbon exposure, a surrogate for diesel exhaust in the trucking industry.

Study subjects were long-term Teamsters enrolled in the pension system who died during the period 1982-1983. Using death certificate information, the researchers identified 994 cases of lung cancer for the study period, and 1,085 non-lung-cancer deaths served as controls. Subjects were divided into job categories based on the job each held the longest. Most had held only one type of job. The job categories were short-haul driver, long-haul driver, mechanic, dockworker,

other jobs with potential diesel exposure, and jobs outside the trucking industry without occupational diesel exposure. Smoking histories were obtained from next of kin. OR 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. OR 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 diesel exhaust exposure, for subjects in the case-control study were made by assuming that average 1990 levels for a job category could be assigned to all subjects in that category, and that levels prior to 1990 were directly proportional to vehicle miles traveled by heavy-duty trucks and the estimated emission levels of diesel engines. A 1975 exposure level of elemental carbon in 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 OR 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 diesel exhaust exposures and dockworkers who mainly used propane-powered forklifts had the lowest exposure. ORs of 1.69 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 diesel exhaust 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 diesel exhaust 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 diesel exhaust among workers in the trucking industry. They assert that these estimates suggest that the lifetime excess risk for lung cancer is 10 times higher than the OSHA standards, but caution that the results should be viewed as exploratory.

The authors acknowledge that the increasing trend in risk with increasing estimates of cumulative exposure is partly due to the fact that a component of cumulative dose is simple duration of exposure, and that analyses by simple duration also exhibit a positive trend with duration. This analysis essentially weights the duration by contrived estimates of exposure intensity, and 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 diesel exhaust and lung cancer incidence. Instead, it is an attempt to convert the data from Steenland's earlier study of lung cancer for the purpose of estimating a different risk metric, “lifetime excess risk of lung cancer,” by augmenting these data with limited industrial hygiene data and rationalizations about plausible models for cumulative exposure.

The Health Effects Institute (HEI, 1999) and others have raised some questions about the exposure estimations and control for confounding variables. EPA and NIOSH will address these concerns in the year 2000. It should be noted that these concerns are about the use of these data for quantitative risk assessment. As far as qualitative risk assessment is concerned, this study is still considered to be positive and strong.

7.2.2.9. *Boffetta et al. (1990): Case-Control Study on Occupational Exposure to Diesel Exhaust and Lung Cancer Risk*

This is an ongoing (since 1969) case-control study of tobacco-related diseases in 18 hospitals (six U.S. cities). Cases comprise 2,584 males with histologically confirmed primary lung cancers. Sixty-nine cases were matched to 1 control, whereas 2,515 were matched to 2 controls. Controls were individuals who were diagnosed with non-tobacco-related diseases. The matching was done for sex, age (±2 years), hospital, and year of interview. The interviews were conducted at the hospitals at the time of diagnosis. In 1985, the occupational section of the questionnaire was modified to include the usual occupation and up to five other jobs as well as duration (in years) worked in those jobs. After 1985, information was also obtained on exposure to 45 groups of chemicals, including diesel exhaust at the workplace or during hobby activities. A priori aggregation of occupations was categorized into low probability of diesel exhaust exposure (reference group), possible exposure (19 occupations), and probable exposure (13 occupations). Analysis was conducted based on "usual occupation" on all study subjects, and any occupation with sufficient cases was eligible for further analysis. In addition, cases enrolled after 1985 for which there were self-reported diesel exhaust exposure and detailed work histories were also analyzed separately.

Both matched and unmatched analyses were done by calculating the adjusted (for smoking and education) relative odds using the Mantel-Haenzael method and calculating the test-based 95% confidence interval using the Miettinen method. Unconditional logistic regression was used to adjust for potential confounders (the PROC LOGIST of SAS). Linear trends for risk were also tested according to Mantel.

Adjusted relative odds for possible and probable exposure groups as well as the truck drivers were slightly below unity, none being statistically significant for the entire study population. Although slight excesses were observed for the self-reported diesel exhaust exposure group and the subset of post-1985 enrollees for highest duration of exposure (for self-reported exposure, occupations with probable exposure, and truck drivers), none was statistically significant. Trend tests for the risk of lung cancer among self-reported diesel exhaust exposure, probable exposure, and truck drivers with increasing exposure (duration of exposure used as surrogate for increasing dose) were nonsignificant too. Statistically significant lung cancer excesses were observed for cigarette smoking only.

The major strength of this study is availability of detailed smoking history. Even though detailed information was obtained for the usual and five other occupations (1985), because it was difficult to estimate or verify the actual exposure to diesel exhaust, 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 diesel exhaust 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 diesel exhaust and occurrence of lung cancer.

7.2.2.10. *Emmelin et al. (1993): Diesel Exhaust 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 diesel exhaust was tested. Both the weighted linear regressions of the adjusted relative odds and the regression coefficients were used to test mortality trends with all three exposure variables.

Exposure to diesel exhaust was assessed indirectly by initially measuring (1) exposure intensity based on exhaust emission, (2) characteristics of the environment in terms of ventilation, and (3) measures of proportion of time in higher exposed jobs. For exhaust emissions, annual diesel fuel consumption at a port was used as the surrogate. For ventilation, the annual proportion of ships with closed or semiclosed holds was used as the surrogate. The proportion of time spent below decks was used as the surrogate for more exposed jobs. Although data were collected for all three measures, only the annual fuel consumption was used for analysis. Because every man was likely to rotate through the various jobs, the authors thought using annual consumption of diesel fuel was the appropriate measure of exposure. Consequently, in a second analysis, the annual fuel consumption was divided by the number of employees in the same port that year to come up with the fuel-per-person measure, which was further used to create a second measure, “exposed time.” The “annual fuel” and exposed-time data were entered in a calendar time-exposure matrix for each port, from which individual exposure measures were created. A third measure, “machine time” (years of employment from first exposure), was also used to

compare the results with other studies. All exposure measures were accumulated from the first year of employment or first year of diesel machine use, whichever came later. The last year of exposure was fixed at 1979. All exposures up to 2 years before the date of lung cancer diagnosis were omitted from both cases and matched controls. A priori classification into three categories of low, medium, and high exposure was done for all three exposure variables: machine time, fuel, and exposed time.

Conditional logistic regression models, adjusting for smoking status and using low exposures and/or nonsmokers as a comparison group, yielded positive trends for all exposure measures, but no trend test results were reported, and only the relative odds for the exposed-time exposure measure in the high-exposure group (OR = 6.8, 90% CI = 1.3 to 34.9) was reported as statistically significant. For smokers, adjusting for diesel exhaust exposure level, the relative odds were statistically significant and about equal for all three exposure variables: machine time, OR = 5.7 (90% CI = 2.4 to 13.3); fuel, OR = 5.5 (90% CI = 2.4 to 12.7); and exposed time, OR = 6.2 (90% CI = 2.6 to 14.6). Interaction between diesel exhaust and smoking was tested by conditional logistic regression in the exposed-time variable. Although there were positive trends for both smokers and nonsmokers, the trend for smokers was much steeper: low, OR = 3.7 (90% CI = 0.9 to 14.6); medium, OR = 10.7 (90% CI = 1.5 to 78.4); and high, OR = 28.9 (90% CI = 3.5 to 240), indicating more than additive interaction between these two variables.

In the weighted linear regression model with the exposed-time variable, the results were similar to those using the logistic regression model. The authors also explored the smoking variable further in various analyses, some of which suggested a strong interaction between diesel exhaust and smoking. However, with just six nonsmokers and no further categorization of smoking amount or duration, these results are of limited value.

The diesel exhaust exposure matrices created using three different variables are intricate. Analyses by any of these variables yield essentially the same positive results and positive trends, providing consistent support for a real effect of diesel exhaust exposure, at least in smokers. However, methodological limitations to this study prevent a more definitive conclusion. The numbers of cases and controls are small. There are very few nonsmokers; thus, testing the effects of diesel exhaust exposure in them is futile. Lack of information on asbestos exposure, to which dockworkers are usually exposed, may also confound the results. Also, no latency analyses are presented. Overall, despite these limitations, this study supports the earlier findings of excess lung cancer mortality among individuals exposed to diesel exhaust.

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 diesel exhaust 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 ($p \leq 0.5$). 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 diesel exhaust 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 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 OR 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 personal communication with Dr. Johnni Hansen confirmed that lorries, buses, and taxis have been using diesel fuel since the beginning of the 1960s. 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 diesel exhaust 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 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 $\leq$1945, 1946-1955, and $\geq$1956 while the last year of exposure was defined as $\leq$1965, 1966-1975, and $\geq$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. OR 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 ($\leq$1945, 1946-1955, and $\geq$1956) and end year of exposure ($\leq$1965, 1966-1975, and $\geq$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 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 diesel exhaust and occurrence of lung cancer.

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

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

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

The Health Effects Institute (HEI) reviewed all published epidemiologic studies on the health effects of exposure to diesel exhaust available through June 1993, identified by a MEDLINE search and by reviewing the reference sections of published research and earlier reviews. HEI identified 35 reports of epidemiologic studies (16 cohort and 19 case-control) of the relation of occupational exposure to diesel emissions and lung cancer published between 1957 and 1993.

HEI reviewed the 35 reports for epidemiologic evidence of health effects of exposure to diesel exhaust for lung cancer, other cancers, and nonmalignant respiratory disease. They found that the data were strongest for lung cancer. The evidence suggested that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding caused by cigarette smoking or other known sources of bias.

Control for smoking was identified in 15 studies. Six studies (17%) reported relative risk estimates less than 1; 29 studies (83%) reported at least relative risk indicating positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals, which excluded unity.

The authors conclude that epidemiologic data consistently show weak associations between exposure to diesel exhaust and lung cancer. They find that the evidence suggests that long-term exposure to diesel exhaust in a variety of occupational circumstances is associated with a 1.2- to 1.5-fold increase in the relative risk of lung cancer compared with workers classified as unexposed. Most of the studies that controlled for smoking found that the association between increased risk of lung cancer and exposure to diesel exhaust persisted after such controls were applied, although in some cases the excess risk was lower. None of the studies measured exposure to diesel emissions or characterized the actual emissions from the source of exposure for the time period most relevant to the development of lung cancer. Most investigators classified exposure on the basis of work histories reported by subjects or their next of kin, or by retirement records. Although these data provide relative rankings of exposure, the absence of concurrent exposure information is the key factor that limits interpretation of the epidemiologic findings and subsequently their utility in making quantitative estimates of cancer risks.

This is a comprehensive and thorough narrative review of studies of the health effects of diesel exhaust. It does not undertake formal estimation of summary measures of effect or evaluation of heterogeneity in the results. The conclusion drawn about the consistency of the results is based on the author's assessment of the failure of potential biases and alternative explanations for the increase in risk to account for the observed consistency. In many if not most studies, the quality of the data used to control confounding was relatively crude. Although the studies do include qualitative assessment of whether control for smoking is taken into account, careful scrutiny of the quality of the control or adjustment for smoking among the studies is absent. This leaves open the possibility that prevalent residual confounding by inadequate control for smoking in many or most studies may account for the consistent associations seen.

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

Bhatia et al. (1998) report a meta-analysis of 29 published[1] cohort and case-control studies of the relation between occupational exposure to diesel exhaust and lung cancer. A search of the epidemiologic literature was conducted for all studies concerning lung cancer and diesel exhaust exposure. Occupational studies involving mining were excluded because of concern about the possible influence of radon and silica exposures. Studies in which the minimum interval from time of first exposure to end of 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 diesel exhaust-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 diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons. (See Figure 7-1.)

Bhatia and colleagues conclude that the analysis shows a small but consistent increase in the risk for lung cancer among workers with exposure to diesel exhaust. The authors evaluate the dependence of the relative risk estimate on the presence of control for smoking among studies,

[1]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.

and provide a table that allows assessment of whether the quality of the data contributing to control for smoking is related to the relative risk estimates (albeit in a limited number of studies). Bhatia et al. assert that residual confounding is not affecting the summary estimates or conclusions for the following reasons: (1) the pooled relative risks for studies adjusted for smoking were the same as those for studies not adjusting for smoking; (2) in those studies giving risk estimates adjusted for smoking and risk estimates not adjusted for smoking, there was only a small reduction in the pooled relative risk from diesel exhaust exposure; and (3) in studies with internal comparison populations, in which confounding is less likely, the pooled relative risk estimate was 1.43.

The validity of this assessment depends on the adequacy of control for smoking in the individual studies. If inadequate adjustment for smoking is employed and residual confounding by cigarette smoking pertains in the result of the individual studies, then the comparisons and contrasts of the pooled estimates 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 analysis for the 1987 report by Garshick et al. as a continuous variable representing pack-years of smoking, whereas the analysis actually dichotomized the pack-years data into two crude dose categories (above and below the 50 pack-years level). This clearly reduced the quality of the adjustment for smoking, which already suffered from the fact that information on cumulative cigarette consumption was missing for more than 20% of the lung cancer cases. In this instance, the consistency between the adjusted and unadjusted estimates of the relative risk for diesel exhaust exposure may be attributable to failure of adjustment rather than lack of confounding by cigarette smoking, and pooled estimates of association of diesel exhaust with lung cancer derived in the meta-analysis would remain confounded. A similar problem exists for the Bhatia et al. representation of the control for confounding in the study by Boffetta and Stellman (1988). Such mischaracterizations may indicate an overstatement by Bhatia et al. that the association of DE and lung cancer is insensitive to adjustment.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility of publication bias.

7.2.3.3. *Lipsett and Campleman (1999): Occupational Exposure to Diesel Exhaust and Lung Cancer: A Meta-Analysis*

Lipsett and Campleman (1999) conducted electronic searches to identify epidemiologic studies published between 1975 and 1995 of the relationship of occupational exposure to diesel exhaust and lung cancer. Studies were selected based on the following criteria: (1) Estimates of

relative risks and their standard errors must be reported or derivable from the information presented. (2) Studies must have allowed for a latency period of 10 or more years for development of lung cancer after onset of exposure. (3) No obvious bias resulted from incomplete case ascertainment in 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 populations most likely to have had substantial exposure to diesel exhaust, the pooled smoking-adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). (See Figure 7-2.)

The between-study variance of the relative risks indicated the presence of significant heterogeneity in the individual estimates. The authors evaluated the potential sources of 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.

An evaluation of the potential for publication bias is presented that provides reassurance that the magnitude of published effects is not a function of the precision or study power; however, this assessment cannot rule out the possibility of publication bias.

Although a relatively technical approach was used in deriving summary estimates of relative risk and the evaluation of possible sources of variation in the relative risks in this meta-analysis, this approach should not be confused with rigorous evaluation of the potential weaknesses among the studies included in the analysis. The heterogeneity attributable to statistical adjustment for smoking was evaluated on the basis of a dichotomous assessment of whether control for smoking could be identified in the studies considered. This does not reflect the adequacy of the adjustment for smoking employed in the individual studies considered. The

potential for residual confounding by inadequate adjustment for the influence of smoking remains in the summary estimate of the relative risk.

7.2.4. Summary and Discussion

Certain extracts of diesel exhaust have been demonstrated as both mutagenic and carcinogenic in animals and in humans. Animal data suggest that diesel exhaust is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods of time. While rat lung cancer response to diesel exhaust 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 diesel exhaust and because nonoccupational ambient exposures currently are of concern as well, the possibility that exposure to this complex mixture may be carcinogenic to humans has become an important public health issue.

Because diesel emissions become diluted in the ambient air, it is difficult to study the health effects in the general population. Nonoccupational exposure to diesel exhaust is worldwide in urban areas. Thus, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are nonoccupationally exposed to diesel exhaust. Furthermore, the "exposed" group in these studies is based on job titles, which in most instances are not verified or correlated with environmental hygiene measurement. The issue of health effect measurement is further complicated by the fact that occupational cohorts tend to be healthy and have below-average mortality, usually referred to as the "healthy worker effect." Hence, the usual standard mortality ratios observed in cohort mortality studies are likely to be underestimations of true risk.

A major difficulty with the occupational studies considered here was measurement of actual diesel exhaust exposure. Because all the cohort mortality studies were retrospective, assessment of health effects from exposure to diesel exhaust was naturally indirect. In these occupational settings, no systematic quantitative records of ambient air were available. Most studies compared men in job categories with presumably some exposure to diesel exhaust with either standard populations (presumably no exposure to diesel exhaust) or men in other job categories from industries with little or no potential for diesel exhaust exposure. A few studies have included measurements of diesel fumes, but there is no standard method for the measurement. No attempt is made to correlate these exposures with the cancers observed in any of these studies, nor is it clear exactly which extract should have been measured to assess the occupational exposure to diesel exhaust. All studies have relied on the job categories or self-report of exposure to diesel exhaust. Gustavsson et al. (1990), Emmelin et al. (1993), and Brüske-Hohlfeld et al. (1999) estimated exposure levels by getting detailed histories of job tskas/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 on the basis of an industrial hygiene survey done by Woskie et al. (1988a,b). The investigators found that in most cases the job titles were good surrogates for diesel exhaust exposure. Also, in the railroad industry, where only persons who had at least 10 years of work experience were included in the study, the workers tended not to change job categories over the years. Thus, a job known only at one point in time was a reasonable marker of past diesel exhaust exposure. Unfortunately, the exposure was only qualitatively verified. Quantitative use of this information would have been much more meaningful. 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 diesel exhaust are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators. No known studies in metal miners have assessed whether diesel exhaust is associated with lung cancer. Currently, there are about 385 underground metal mines in the United States. Of these, 250 have been permanently operating and 135 have been intermittently operating (Steenland, 1986). Approximately 20,000 miners are employed, but not all of them are currently working in the mines. Diesel engines were introduced in metal mines in the early to mid-1960s. Although all these mines use diesel equipment, it is difficult to estimate how many of these miners were actually exposed to diesel fumes.

Diesel engines were introduced in coal mines at an even later date, and their use is still quite limited. In 1983, approximately 1,000 diesel units were in place in underground coal mines, up from about 200 units in 1977 (Daniel, 1984). The number of units per mine varies greatly; 1 mine may account for more than 100 units.

Even if it were possible to estimate how many miners (metal and coal) were exposed to diesel exhaust, it would be very difficult to separate out the confounding effects of other potential pulmonary carcinogens, such as radon decay products or heavy metals (e.g., arsenic, chromium). Furthermore, the relatively short latency period limits the usefulness of these cohorts of miners.

7.2.4.1. *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 diesel exhaust. Although the Waller (1981) study had a 25-year follow-up period, the cohort was restricted to employees (ages 45 to 64) currently in service. Employees who left the job earlier, as well as those who were still employed after age 64 and who may have died from cancer, were excluded.

Wong et al. (1985) conducted a mortality study of heavy equipment operators that demonstrated a nonsignificant positive trend for cancer of the lung with length of membership and latency. Analysis of deceased retirees showed a significant excess of lung cancer. Individuals without work histories who started work prior to 1967, when records were not kept, may have been in the same jobs for the longest period of time. Workers without job histories included those who had the same job before and after 1967 and thus may have worked about 12 to 14 years longer; these workers exhibited significant excess risks of lung cancer and stomach cancer. If this assumption about duration of jobs is correct, then these site-specific causes can be linked to diesel exhaust exposure. One of the methodologic limitations of this study is that most of these men worked outdoors; thus, this cohort might have had relatively low exposure to diesel exhaust. The authors did not present any environmental measurement data either. Because of the absence of detailed work histories for 30% of the cohort and the availability of only partial work histories for the remaining 70%, jobs were classified and ranked according to presumed diesel exposure. Information is lacking regarding duration of employment in the job categories (used for surrogate of exposure) and other confounding factors (alcohol consumption, cigarette smoking, etc.). Thus, this study cannot be used to support or refute a causal association between exposure to diesel exhaust and lung cancer.

A 2-year mortality analysis by Boffetta and Stellman (1988) of the American Cancer Society's prospective study, after controlling for age and smoking, demonstrated an excess risk of lung cancer in certain occupations with potential exposure to diesel exhaust. These excesses were statistically significant among miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.6, 95% CI = 1.12, 6.06). 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 diesel exhaust occurred in the three other occupations showing an increase in the risk of lung cancer. Despite methodologic limitations, such as the lack of representiveness of the study population (composed of volunteers only, who were probably healthier than the general population), leading to an underestimation of the risk, and the questionable reliability of exposure

data based on self-administered questionnaires that were not validated, this study is suggestive of a causal association between exposure to diesel exhaust and excess risk of lung cancer.

Two mortality studies were conducted by Gustavsson et al. (1990) and Hansen (1993) among bus garage workers (Stockholm, Sweden) and truck drivers, respectively. An SMR of 122 was found among bus garage workers, based on 17 cases. A nested case-control study was also conducted in this cohort. Detailed exposure matrices based on job tasks were assembled for both diesel exhaust and asbestos exposures. Statistically significant increasing lung cancer relative risks of 1.34, 1.81, and 2.43 were observed for diesel exhaust indices of 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as a comparison group. Adjustment for asbestos exposure did not change the results. The main strength of this study is the detailed exposure matrices; some of the limitations are 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 diesel exhaust.

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 diesel exhaust 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 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 diesel exhaust 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 diesel fumes. Information on jobs was available at retirement only. There also was insufficient detail on the classification of jobs by diesel exhaust exposure. The exposures could have been nonconcurrent or concurrent, but because the data are lacking, it is possible that the observed excess could be due to the effect of both coal dust/combustion fumes and diesel 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 diesel exhaust exposure categories were examined for smoking-related diseases such as emphysema, laryngeal cancer, esophageal cancer, and buccal cancer, positive trends were observed, raising a possibility that the dose response demonstrated for diesel exposure may have been due to smoking. The findings of this study are at best suggestive of diesel exhaust being a lung carcinogen.

The strong evidence for linking diesel exhaust exposure to lung cancer comes from the Garshick et al. (1988) railroad worker study conducted in the United States. Relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) were found for ages 40 to 44 and 45 to 49, respectively, after the exclusion of workers exposed to asbestos. 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 diesel exhaust 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. (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 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 a negative dose response in his latest analysis. The divergent results of these recent analyses do not negate the strong 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 from occupational histories. Next of kin provided the occupational histories for 50% of the cases that were not validated. The power of the study was small (analysis done on males only, 333 cases). Similarly, Boffeta et al. (1990) did not find any excess of lung cancer after adjusting for smoking and education. This study had a few methodological limitations. The lung cancer cases and controls were drawn from the ongoing study of tobacco-related diseases. It is interesting to note that the leading risk factor for lung cancer is cigarette smoking. The exposure was not measured. Instead, occupations were used as surrogates for exposure. Furthermore, there were very few individuals in the study who were exposed to diesel exhaust. On the other hand, statistically nonsignificant excess risks were observed for diesel exhaust exposure by Hall and Wynder (1984) in workers who were exposed to diesel exhaust versus those who were not (OR = 1.4 and 1.7 with two different criteria) and by Damber and Larsson (1987) in professional drivers (OR = 1.2). These rates were adjusted for age and smoking. 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 diesel exhaust 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 diesel exhaust. They found that truck drivers employed for more than 10 years had a significantly increased risk of lung cancer (OR = 1.5, 95% CI = 1.1, 1.9). This study also found a significant trend of increasing risk of lung cancer with increasing duration of employment among truck drivers. The relative odds were computed by adjusting for birth cohort, smoking, and State of residence. The main limitation of this study is again the mixed exposures to diesel and gasoline exhausts, because information on type of engine was lacking. Also, potential bias may have been introduced because the way in which the cause of death was ascertained for

the selection of cases varied in the three studies. Furthermore, the methods used in these studies to classify occupational categories were different, probably leading to incompatibility of occupational categories.

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 diesel exhaust was 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 diesel exhaust exposure and occurrence of lung cancer, at least in smokers.

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 $\geq$20 years and railroad workers employed for $\geq$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 $\geq$20 years (OR = 10.4, 95% CI = 1.4, 77.1). Although individual ORs were not significant for various occupations with potential exposure to diesel exhaust, statistically significant trends were observed for drivers of heavy trucks, light trucks, farmers, and railroad industry workers among whites, and among black farmers ($p \leq 0.5$). 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 diesel exhaust of their farm tractors.

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 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. found that after adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95% CI = 1.05, 1.83). Among the younger workers with longer diesel exhaust exposure, the risk of lung cancer increased with duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent diesel exhaust exposure (5 years before death), the relative odds increased to 1.43 (95% CI = 1.06, 1.94). This appears to be a well-conducted and well-analyzed study with reasonably good power. Potential confounders were controlled adequately, and interactions between diesel exhaust and other lung cancer risk factors were tested. Some of the limitations of this study are inadequate latency period, 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 highest current levels of diesel exhaust 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 diesel exhaust exposure and development of lung cancer. However, the risk among mechanics did not increase with increasing duration of employment. The OR for quartile cumulative exposure, 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.

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 early 1960s. 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.

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. 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.

The main limitation of the study is lack of data on actual exposure to DME. The cumulative quantitative exposures were calculated on the basis of 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 diesel exhaust 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 diesel exhaust exposure and lung cancer risk are reviewed. The HEI report is a narrative study of more than 35 epidemiologic studies (16 cohort and 19 case-control) of occupational exposure to diesel emissions published between 1957 and 1993. Control for smoking was identified in 15 studies. Six of the studies (17%) reported relative risk estimates less than 1, whereas 29 (83%) reported at least 1 relative risk, indicating a positive association. Twelve studies indicating a relative risk greater than 1 had 95% confidence intervals that excluded unity. These studies found that the evidence suggests that occupational exposure to diesel exhaust from diverse sources increases the rate of lung cancer by 20% to 40% in exposed workers generally, and to a greater extent among workers with prolonged exposure. They also found that the results are not explicable by confounding due to cigarette smoking 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 diesel exhaust. Subanalyses by study design (case-control and cohort studies) and by control for smoking produced results that did not differ from those of the overall pooled analysis. Cohort studies using internal comparisons showed higher relative risks than those using external comparisons.

Lipsett and Campleman (1999) identify 39 independent estimates of relative risk among 30 eligible studies of diesel exhaust and lung cancer published between 1975 and 1995. Pooled relative risks for all studies and for study subsets were estimated using a random effect model. Interstudy heterogeneity was also modeled and evaluated. A pooled smoking-adjusted relative risk was 1.47 (95% CI = 1.29, 1.67). Substantial heterogeneity was found in the pooled-risk estimates. Adjustment for confounding by smoking, having a lower likelihood of selection bias, and increased study power were all found to contribute to lower heterogeneity and increased pooled estimates of relative risk.

There is some variability in the conclusions of these summaries of the association of diesel exhaust and lung cancer. The three analyses find that smoking is unlikely to account for the observed effects, and all conclude that the data support a causal association between lung cancer and diesel exhaust exposure. On the other hand, Stöber and Abel (1996), Muscat and Wynder (1995), and Cox (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 diesel exhaust and lung cancer are unlikely to be due to bias. They argue that methodologic problems are prevalent among the studies, especially in evaluation of diesel engine exposure and control of confounding by cigarette smoking. The conclusions of the two meta-analyses are based on magnitude of pooled relative risk estimates and evaluation of potential sources of heterogeneity in the estimates. Despite the statistical sophistication of the meta-analyses, the statistical models used cannot compensate for deficiencies in the original studies and will remain biased to the extent that bias exists in the original studies.

7.2.4.4. *Discussion of Relevant Methodologic Issues*

A persistent association of risk for lung cancer and diesel exhaust 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 diesel exhaust 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 diesel exhaust and lung cancer risk in so many studies indicates that the possibility is remote that the observed association in aggregate is due to chance. It is unlikely that chance alone accounts for the observed relation between diesel exhaust and lung cancer.

The excess risk is observed in both cohort and case-control designs, which contradicts the concern that a methodologic bias specifically characteristic of either design (e.g., recall bias) might account for the observed effect. Selection bias is certainly present in some of the

occupational cohort studies that use external population data in estimating relative risks, but this form of selection bias (a healthy worker effect) would only obscure, rather than spuriously produce, an association between diesel exhaust and lung cancer. Several occupational epidemiologic studies that use more appropriate data for their estimates are available. Selection biases may be operating in some case-control studies, but it is not obvious how such a bias could be sufficiently uniform in effect, prevalent, and strong enough to lead to the consistent association seen in the aggregate data. Given the variety of designs used in studying the diesel exhaust and lung cancer association and the number of studies in different populations, it is unlikely that routinely studying noncomparable groups is an explanation for the consistent association seen. Exposure information bias is certainly a problem for almost all of the studies concerned. Detailed and reliable individual-level data on diesel exhaust exposure for the period of time relevant to the induction of lung cancer are not available and are difficult to obtain. Generally, the only information from which diesel exposure can be inferred is occupational data, which is a poor surrogate for the true underlying exposure distribution. Study endpoints are frequently mortality data taken from death certificate information, which is frequently inaccurate and often does not fully characterize the lung cancer incidence experience of the population in question. Using inaccurate surrogates for lung cancer incidence and for diesel exposure can lead to substantial bias, and these shortcomings are endemic in the field. In most cases these shortcomings will lead to misclassification of exposure and of outcome, which is nondifferential. Nondifferential misclassification of exposure and/or outcome can bias estimates of a diesel exhaust–lung cancer association, if one exists, toward the null; but it is unlikely that such misclassification would produce a spurious estimate in any one study. It is even more unlikely that it would bias a sufficient number of studies in a uniform direction to account for the persistent aggregate association observed.

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 al. found that the age at which an individual started smoking was reported within 4 years of actual age 84% of the time. These studies indicate that surrogates were able to provide fairly credible information on the smoking habits of the study subjects. If the surrogates of the cases were more likely to overreport cigarette smoking compared with the controls, then it might be harder to find an effect of diesel exhaust because most of the increase in lung cancer would be attributed to smoking rather than to exposure to diesel exhaust.

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 diesel exhaust 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 diagnosed on the death certificate.

Finally, several investigators have not conducted latency analysis in their studies. The latent period for lung cancer development is up 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 USA, most of the cohort studies do not have a long enough follow-up period to allow for latency of 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 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. 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 diesel exhaust 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 diesel exhaust. 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 early 1960s.

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 provided a set of criteria that define a causal relationship between exposure and the health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually proves causality; actual proof is rarely attainable when dealing with environmental carcinogens. None of these criteria should be considered either necessary (except temporality of exposure) or sufficient in itself. The absence of any one or even several of these criteria does not prevent a causal interpretation. However, if more criteria apply, this provides more credible evidence for causality.

Thus, applying the 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 et al., 1983; Wong et al., 1985; Gustavsson et al., 1990; Emmlin et al., 1993; Hansen et al., 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 diesel exhaust 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 diesel exhaust. 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 et al., 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 et al., 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 diesel exhaust, 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 diesel exhaust for other effects such as bladder cancer, leukemia, gastrointestinal cancers, prostate cancer etc. The evidence for these effects is inadequate. Rothman (1986), in his discussion about causality criteria, states “Causes of a given effect, however, cannot be expected to be without other effects on any logical grounds. In fact, everyday experience teaches us repeatedly that single events may have many effects. Hill’s discussion of this standard for inference is replete with reservations, but even so, the criterion seems useless and misleading.”
- *Temporality*. The only necessary, but not sufficient, criterion described by Hill for causality inference is that exposure to a causal agent precedes the effect in time. This criterion is clearly satisfied in the studies reviewed here. Temporality can be explored further in addressing the latency issue. A certain period is necessary for

development of an effect after exposure to a causal agent has occurred. For instance, in cancer-causing agents a latent period can vary from 5 years (childhood leukemia) to ≥30 years (mesothelioma). Most of the studies reviewed here did not conduct the latency analysis. Some studies had a short follow-up period that did not allow enough time for the latency period (Waller, 1981; Howe et al., 1983; Rushton et al., 1983; Wong et al., 1985, Hansen et al., 1993) while several studies clearly allowed for an adequate latency period (Garshick et al., 1987; Gustavsson et al., 1990; Steenland et al., 1990; Swanson et al., 1993; Brüske-Hohlfeld et al., 1999). Both type of studies showed mixed results.

- *Biological gradient.* This criterion refers to the dose-response curve. Due to the lack of quantitative data on diesel exhaust exposure in most studies reviewed here, analyzing the dose-response curve directly was not possible. In very few studies was exposure to diesel exhaust addressed specifically. Most investigators have used job titles/categories and duration of employment as surrogates for exposure and thus have presented response in relation to duration of employment. Significant dose-response (using duration of employment as a surrogate) was observed in various studies for railroad workers (Howe et al., 1983; Garshick et al., 1987; Garshick et al., 1988; Swanson et al., 1993), truck drivers (Boffetta and Stellman, 1988; Hayes et al., 1989; Steenland et al., 1990; Swanson et al., 1993; Hansen et al., 1998; Brüske-Hohlfeld et al., 1999), transportation/heavy equipment operators (Wong et al., 1985; Gustavsson et al., 1990; Brüske-Hohlfeld et al., 1999), farmers/farm tractor users (Swanson et al., 1993; Brüske-Hohlfeld et al., 1999), and dockworkers (Emmelin et al., 1993).
- *Biological plausibility.* This criterion refers to the biologic plausibility of the hypothesis, an important concern that may be difficult to judge. The hypothesis considered for this review is that occupational exposure to diesel exhaust is causally associated with the occurrence of lung cancer and is supported by the following: First, diesel exhaust 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 et al., 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, diesel exhaust 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

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 diesel exhaust have been observed in humans (Nielsen et al., 1996a,b). Thus, the above evidence makes a convincing case that occupational exposures to diesel exhaust are causally associated with the occurrence of lung cancer—highly plausible biologically.

In conclusion, the epidemiologic studies of exposure to diesel exhaust 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, confounding by smoking is unlikely in these studies because the comparison population was from the same socioeconomic class. The strength of association was weak to modest (RRs/ORs between 1.2 and 2.6), with dose-response relationship observed in several studies. Last, but not least, there is strong evidence for biological plausibility that exposure to diesel exhaust would result in excess risk of lung cancer in humans.

7.3. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. The first portion of this chapter summarizes results of inhalation studies. Experimental protocols for the inhalation studies typically consisted of exposure (usually chronic) to diluted exhaust in whole-body exposure chambers using rats, mice, and hamsters as model species. Some of these studies used both filtered (free of particulate matter) diesel exhaust and unfiltered (whole) diesel exhaust to differentiate gaseous-phase effects from effects induced by 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 diesel exhaust and tumor initiators, tumor promoters, or cocarcinogens.

Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 mg/m^3. In this chapter, any indication of statistical significance implies that $p \leq 0.05$ was reported in the reviewed publications. 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 diesel exhaust was first evaluated by Karagianes et al. (1981). Male Wistar rats (40 per group) were exposed to room air or diesel engine exhaust diluted to a DPM concentration of 8.3 (± 2.0) mg/m^3, 6 hr/day, 5 days/week for up to 20 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^o and 26.5 ^{o}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 diesel exhaust. 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.

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 diesel exhaust 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 diesel exhaust 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 diesel exhaust to induction of lung cancer in rats were reported by researchers in Germany, Switzerland, Japan, and the United States in the mid-to-late 1980s. In a study conducted at the Fraunhofer Institute 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) diesel exhaust at an average particulate matter concentration of 4.24 mg/m^3. Animals were exposed for a maximum of 2.5 years. The exposure system as described by Heinrich et al. (1986a) used a 40 kilowatt 1.6-L diesel engine operated continuously under the U.S. 72 FTP driving cycle. The engines used European Reference Fuel with a sulfur content of 0.36%. Filtered exhaust was obtained by passing engine exhaust through a Luwa FP-65 HT 610 particle filter heated to 80 °C and a secondary series of filters (Luwa FP-85, Luwa NS-30, and Drager CH 63302) at room temperature. The filtered and unfiltered exhausts were diluted 1:17 with filtered air and passed through respective 12 m^3 exposure chambers. Mass median aerodynamic diameter of DPM was 0.35 ± 0.10 µm (mean ± SD). The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A.

The effects of exposure to either filtered or unfiltered exhaust were described by Heinrich et al. (1986b) and Stöber (1986). Exposure to unfiltered exhaust resulted in 8 bronchoalveolar adenomas and 9 squamous cell tumors in 15 of 95 female Wistar rats examined, for a 15.8% tumor incidence. Although statistical analysis was not provided, the increase appears to be highly significant. In addition to the bronchioalveolar adenomas and squamous cell tumors, there was a high incidence of bronchioalveolar hyperplasia (99%) and metaplasia of the bronchioalveolar epithelium (65%). No tumors were reported among rats exposed to filtered exhaust (n = 92) or clean air (n = 96).

Mohr et al. (1986) provided a more detailed description of the lung lesions and tumors identified by Heinrich et al. (1986a,b) and Stöber (1986). Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to unfiltered diesel exhaust. Squamous

metaplasia was observed in 65.3% of the rats breathing unfiltered diesel exhaust, but not in the control rats. Of nine squamous cell tumors, one was characterized as a Grade I carcinoma (borderline atypia, few to moderate mitoses, and slight evidence of stromal invasion), and the remaining eight were classified as benign keratinizing cystic tumors.

Iwai et al. (1986) examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 2.4-L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20 °C to 25 °C and 50% relative humidity. The engines were operated at 1,000 rpm with an 80% engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest NO_2 and SO_2 content. For those chambers using filtered exhaust, proximally installed high-efficiency particulate air (HEPA) filters were used. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air for 8 hr/day, 7 days/week for 24 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 diesel exhaust. Nineteen of the 24 exposed to unfiltered exhaust survived for 2 years. Of these, 14 were randomly selected for sacrifice at this time. Four of the rats developed lung tumors; two of these were malignant. Five rats of this 2-year exposure group were subsequently placed in clean room air for 3 to 6 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 diesel exhaust. The tumor types identified were adenoma (3/19), adenocarcinoma (1/19), adenosquamous carcinoma (2/19), squamous carcinoma (1/19), and large-cell carcinoma (1/19). The lung tumor incidence in rats exposed to whole diesel exhaust was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-3. 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 diesel exhaust. In addition, splenic malignant lymphomas occurred during exposure to both filtered and unfiltered diesel exhaust. This is the only report to date of tumor induction at an extrarespiratory site by inhaled diesel exhaust in animals.

A chronic (up to 24 months) inhalation exposure study was conducted by Takemoto et al. (1986), in which female Fischer 344 rats were exposed to diesel exhaust generated by a 269-cc YANMAR-40CE NSA engine operated at an idle state (1,600 rpm). Exposures were 4

hours/day, 4 days/week. The animals were exposed in a 376-L exposure chamber. Air flow was maintained at 120 L/min. Exhaust was diluted to produce a particle concentration of 2-4 mg/m^3. When not exposed the animals were maintained in an air-conditioned room at a temperature of 24 ± 2°C and a relative humidity of 55 ± 5% with 12 hr of light and darkness. Temperature and humidity in the exposure chambers was not noted. The particle concentration of the diesel exhaust in the exposure chamber was 2 to 4 mg/m^3. B[a]P and 1-nitropyrene concentrations were 0.85 and 93 µg/g of particles, respectively. No lung tumors were reported in the diesel-exposed animals. It was also noted that the diesel engine employed in this study was originally used as an electrical generator and that its operating characteristics (not specified) were different from those 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 diesel exhaust in rats following chronic inhalation exposure. In addition, increasing lung particle burden resulting from this high-level exposure and decreased clearance was demonstrated. A logistic regression model presented by Mauderly et al. (1987) indicated that both lung DPM burden and exposure concentration may be useful for expressing exposure-effect relationships.

A long-term inhalation study (Ishinishi et al., 1988a; Takaki et al., 1989) examined the effects of emissions from a light-duty (LD) and a heavy-duty (HD) diesel engine on male and female Fischer 344/Jcl rats. The LD engines were 1.8-L, 4-cylinder, swirl-chamber-type power plants, and the HD engines were 11-L, 6-cylinder, direct-injection-type power plants. The engines were connected to eddy-current dynamometers and operated at 1,200 rpm (LD engines) and 1,700 rpm (HD engines). Nippon Oil Co. JIS No. 1 or No. 2 diesel fuel was used. The 30-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

72 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 diesel exhaust (DPM concentration of 0.1 or 1.1 mg/m^3 for the LD engine and 0.5 or 1.8 mg/m^3 for the HD engine) for 12 months were examined for lung tumors following 6-, 12-, or 18-months recovery periods in clean air. The incidences of neoplastic lesions were low, and pulmonary DPM burden was lower than for animals continuously exposed to whole diesel exhaust and not provided a recovery period. The only carcinoma observed was in a rat examined 12 months following exposure to exhaust (1.8 mg/m^3) from the HD engine.

Brightwell et al. (1986, 1989) studied the effects of diesel exhaust on male and female F344 rats. The diesel exhaust was generated by a 1.5-L Volkswagen engine that was computer-operated according to the U.S. 72 FTP driving cycle. The engine was replaced after 15 mo. The engine emissions were diluted by conditioned air delivered at 800 m^3/h to produce the high-exposure (6.6 mg/m^3) diesel exhaust atmosphere. Further dilutions of 1:3 and 1:9 produced the medium- (2.2 mg/m^3) and low- (0.7 mg/m^3) exposure atmospheres. The CO and NO_x concentrations (mean ± SD) were 32 ± 11 ppm and 8 ± 1 ppm in the high-exposure concentration chamber. The inhalation exposures were conducted overnight to provide five 16-h periods per week for 2 years; surviving animals were maintained for an additional 6 mo.

For males and females combined, a 1.2% (3/260), 0.7% (1/144), 9.7% (14/144), and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to clean air or 0.7, 2.2, and 6.6 mg of DPM/m^3, respectively (Table 7-3). Diesel exhaust-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 diesel exhaust atmospheres. When tumor incidence is expressed relative to the specific intervals, a lung tumor incidence of 96% (24/25), 76% (19/25) of which were malignant, was reported for female rats in the high-dose group exposed for 24 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 diesel exhaust included adenomas (40), squamous cell carcinomas (35), adenocarcinomas (19), mixed adenoma/adenocarcinomas (9), and mesothelioma (1). It should be noted that exposure during darkness (when increased activity would result in greater respiratory exchange and greater inhaled dose) could account, in part, for the high response reported for the rats.

Lewis et al. (1989) also examined the effects of inhalation exposure of diesel exhaust and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole diesel exhaust (2 mg soot/m^3), coal dust (2 mg/m^3 respirable concentration; 5 to 6 mg/m^3 total concentration), or diesel exhaust plus coal dust (1 mg/m^3 of each respirable concentration; 3.2 mg/m^3 total concentration) for 7 h/day, 5 days/week during daylight hours for up to 24 mo. Groups of 10 or more males were sacrificed at intermediate intervals (3, 6, and 12 mo). The diesel exhaust was produced by a 7.0-L, 4-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel (<0.5% sulfur by mass). The exhaust was passed through a Wagner water scrubber, which lowered the exhaust temperature and quenched engine backfire. The animals were exposed in 100-cubic-foot chambers. Temperature was controlled at 22±2 °C and relative humidity at 50%±10%. The exhaust was diluted 27-fold with chemically and biologically filtered clean air to achieve the desired particle concentration.

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 diesel exhaust (0.8, 2.5, or 7.0 mg/m^3) 18 h/day, 5 days/week for up to 24 mo, then held in clean air an additional 6 mo. The animals were exposed in either 6 or 12 m^3 exposure chambers. Temperature and relative humidity were maintined at 23-25 °C and 50%-70%, repectively. Diesel exhaust was generated by two 40-kw 1.6-L diesel engines (Volkswagen). One of them was operated according to the U.S. 72 cycle. The other was operated under constant load conditions. The first engine did not supply sufficient exhaust, which was filled by the second engine. Cumulative exposures for the rats in the various treatment groups were 61.7, 21.8, and 7.4 g/m^3 × h for the high, medium, and low whole-exhaust exposures. Significant increases in tumor incidences were observed in the high (22/100; p<0.001) and mid (11/200; p<0.01) exposure groups relative to clean-air controls (Table 7-3). 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 diesel exhaust. For the high-diesel-exhaust group, 3-mo recovery time in clean air failed to reverse the compromised alveolar clearance.

In a study conducted at the Inhalation Toxicology Research Institute (Nikula et al., 1995) F344 rats (114-115 per sex per group) were exposed 16 hr/day, 5 days/week during daylight hours to diesel exhaust diluted to achieve particle concentrations of 2.5 or 6.5 mg/m^3 for up to 24 mo. Controls (118 males, 114 females) were exposed to clean air. Surviving rats were maintained an additional 6 weeks in clean air, at which time mortality reached 90%. Diesel exhaust was generated 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 diesel exhaust exposure, the lung burdens were 36.7 and 80.7 mg, respectively, for females and 45.1 and 90.1 mg, respectively, for males. The percentages of susceptible rats (males and females combined) with malignant neoplasms were 0.9 (control), 3.3 (low diesel exhaust), and 12.3 (high diesel exhaust). The percentages of rats (males and females combined) with malignant or benign neoplasms were 1.4 (control), 6.2 (low diesel exhaust), and 17.9 (high diesel exhaust). All primary neoplasms were associated with the parenchyma rather than the conducting airways of the lungs. The first lung neoplasm was observed at 15 mo. Among 212 males and females examined in the high-dose group, adenomas were detected in 23 animals, adenocarcinomas in 22 animals, squamous cell carcinomas in 3 animals, and an adenosquamous carcinoma in 1 animal. For further details see Table 7-3. 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 diesel exhaust the following number of tumors were detected; 57 adenomas, 24 adenocarcinomas, 2 benign squamous cell tumors, 7 squamous cell carcinomas, and 3 adenosquamous carcinomas. The authors stated that benign squamous cell tumors probably corresponded to squamous cysts in another classification.

7.3.1.2. *Mouse Studies*

A series of inhalation studies using strain A mice was conducted by Orthoefer et al. (1981). Strain A mice are usually given a series of intraperitoneal injections with the test agent; they are then sacrificed at about 9 months and examined for lung tumors. In the present series, inhalation exposure was substituted. Diesel exhaust was provided by one of two Nissan CN6-33 diesel engines having a displacement of 3244 cc and run on a Federal Short Cycle. Flow through the exposure chambers was sufficient to provide 15 air changes per hour. Temperature was maintained at 24 °C and relative humidity at 75%. In the first study, groups of 25 male Strong A strain (A/S) mice were exposed to irradiated diesel exhaust (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m^3) for 20 h/day, 7 days/week. Additional groups of 40 Jackson A strain (S/J) mice (20 of each sex) were exposed similarly to either clean air or diesel exhaust, then held in clean air until sacrificed at 9 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 diesel exhaust, 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 diesel exhaust generated by a 5.7-L Oldsmobile engine operated continuously at 40 mph at DPM concentrations of 0, 0.25, 0.75, or 1.5 mg/m^3. Controls were exposed to clean air. 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 diesel exhaust. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to diesel exhaust for 8 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 diesel exhaust. Male and female parent-generation mice were exposed to diesel exhaust at a DPM concentration of 6 mg/m^3 prior to (from weaning to sexual maturity) and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) were exposed to either diesel exhaust or clean air. The exhaust exposure was increased to a DPM concentration of 12 mg/m^3 when the offspring were 12 weeks of age and was maintained until termination of the experiment when the mice were 15 months old.

The incidence of pulmonary adenomas (16.3%) was significantly increased in the mice exposed to diesel exhaust compared with 6.3% in clean-air controls. The incidence in males and females combined was 10.2% in 205 animals examined compared with 5.1% in 205 clean-air controls. This difference was also significant. The incidence of carcinomas was not affected by exhaust exposure in either sex. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The increase in the sensitivity of the study, allowing detection of tumors at 15 mo, may have been the result of exposure from conception. It is likely that Sencar mice are sensitive to induction of lung tumors 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 diesel exhaust (2 to 4 mg/m^3, 4 h/day, 4 days/week, for up to 28 mo) in ICR and C57BL mice exposed from birth. Details of the exposure conditions are presented in Section 7.3.2.1. All numbers reported are for males and females combined. Four adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-

exposed ICR mice autopsied at 13 to 18 mo, compared with 3 adenomas among 38 controls. Six adenomas and 3 adenocarcinomas were reported in 22 diesel-exposed ICR mice autopsied at 19 to 28 mo, compared with 3 adenomas and 1 adenocarcinoma in 22 controls. Four adenomas and 2 adenocarcinomas were detected in 79 C57BL mice autopsied at 13 to 18 mo, compared with none in 19 unexposed animals. Among males and females autopsied at 19 to 28 mo, 8 adenomas and 3 adenocarcinomas were detected in 71 exposed animals, compared with 1 adenoma among 32 controls. No significant increases in adenoma or adenocarcinoma were reported for either strain of exposed mice. However, the significance of the increase in the combined incidence of adenomas and carcinomas was not evaluated statistically. A statistical analysis by Pott and Heinrich (1990) indicated that the difference in combined benign and malignant tumors between whole diesel exhaust-exposed C57BL/6N mice and corresponding controls was significant at $p<.05$. See Table 7-3 for details of tumor incidence.

Heinrich et al. (1986b) and Stöber (1986), as part of a larger study, also evaluated the effects of diesel exhaust in mice. Details of the exposure conditions reported by Heinrich et al. (1986a) are given in Section 7.3.1.1 and Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to diesel exhaust diluted to achieve a particle concentration of 4.2 mg/m^3, 76 female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 32%. Tumor incidences reported for control mice (n = 84) equaled 11% for adenomas and adenocarcinomas combined. While the incidence of adenomas showed little change, adenocarcinomas increased significantly from 2.4% for controls to 17% for exhaust-exposed mice. In a follow-up study, however, Heinrich et al. (1995) reported a lack of tumorigenic response in either female NMRI or C57BL/6N mice exposed 17 h/day, 5 days/week for 13.5 to 23 months to whole diesel exhaust 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 diesel exhaust (7.1, 3.5, or 0.35 mg DPM/m^3) for 7 hr/day, 5 days/week for up to 24 mo. Controls were exposed to filtered air. Diesel exhaust was provided by 5.7-L Oldsmobile V-8 engines operated continuously on the U.S. Federal Test Procedure urban certification cycle. The chambers were maintained at 25-28 °C, relative humidity at 40%-60%, and a flow rate sufficient for 15 air exchanges per hour. Animals were exposed during the light cycle, which ran from 6:00 AM to 6:00 PM. DPM accumulation in the lungs of exposed mice was assessed at 6, 12, and 18 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 diesel exhaust exposure on tumor frequency in female Syrian golden hamsters. Groups of 48 to 72 animals were exposed to clean air or whole diesel exhaust at a mean DPM concentration of 3.9 mg/m^3. Inhalation exposures were conducted 7 to 8 hr/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated under a constant load and a constant speed of 2,400 rpm. Flow rate was sufficient for about 20 exchanges per hour in the 250-L chambers. No lung tumors were reported in either exposure group.

In a subsequent study, Syrian hamsters were exposed 19 hr/day, 5 days/week for a lifetime to diesel exhaust diluted to a DPM concentration of 4.24 mg/m^3 (Heinrich et al., 1986b; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air or exhaust. No lung tumors were seen in either the clean-air group or in the diesel exhaust-exposed group.

In a third study (Heinrich et al., 1989b), hamsters were exposed to exhaust from a Daimler-Benz 2.4-L engine operated at a constant load of about 15 kW and at a uniform speed of 2,000 rpm. The exhaust was diluted to an exhaust-clean air ratio of about 1:13, resulting in a mean particle concentration of 3.75 mg/m^3. Exposures were conducted in chambers maintained at 22 to 24 °C and 40% to 60% relative humidity for up to 18 mo. Surviving hamsters were maintained in clean air for up to an additional 6 mo. The animals were exposed 19 hr/day, 5 days/week beginning at noon each day, under a 12-hr light cycle starting at 7 AM. Forty animals per group were exposed to whole diesel exhaust or clean air. No lung tumors were detected in either the clean-air or diesel-exposed hamsters.

Brightwell et al. (1986, 1989) studied the effects of diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females, 6 to 8 weeks old, were exposed to diesel exhaust at DPM concentrations of 0.7, 2.2, or 6.6 mg/m^3. They were exposed 16 hr/day, 5

days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Section 7.3.1.1. No statistically significant (*t* test) relationship between tumor incidence and exhaust exposure was reported.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex in several studies of chronic duration at high exposure concentrations.

7.3.1.4. *Monkey Studies*

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m^3) for 7 hr/day, 5 days/week for 24 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), diesel exhaust plus coal dust (1 mg/m^3 respirable concentration for each component; 3.2 mg/m^3 total concentration), or filtered air. Details of exposure conditions were listed previously in the description of the Lewis et al. (1989) study with rats (Section 7.3.1.1) and are listed in Appendix A.

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo 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 Diesel Exhaust)

Several studies have been conducted in which animals were exposed to diesel exhaust 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., 1986), in Fischer 344 rats of either sex exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 6.6 mg/m^3 (Brightwell et al., 1989), in female Wistar rats exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 7.0 mg/m^3 (Heinrich et al., 1995), and in female Fischer 344 rats exposed to filtered exhaust diluted to produce unfiltered particle concentrations of 5.1, 3.2, or 9.4 mg/m^3 (Iwai et al., 1997). In the Iwai et al. (1986) study, splenic lymphomas were detected in 37.5% of the exposed rats compared with 8.2% in controls.

In the study reported by Heinrich at al. (1986a) and Stober (1986), primary lung tumors were seen in 29/93 NMRI mice (males and females combined) exposed to filtered exhaust, compared with 11/84 in clean-air controls, a statistically significant increase. In a repeat study by Heinrich et al. (1995), however, significant lung tumor increases were not detected in either female NMRI or C57BL/6N mice exposed to filtered exhaust diluted to produce an unfiltered particle concentration of 4.5 mg/m^3.

Filtered exhaust also failed to induce lung tumor induction in Syrian Golden hamsters (Heinrich et al., 1986a; Brightwell et al., 1989).

Although lung tumor increases were reported in one study and lymphomas in another, these results could not be confirmed in subsequent investigations. It is therefore concluded that little direct evidence exists for carcinogenicity of the vapor phase of diesel exhaust in laboratory animals at concentrations tested.

7.3.3. Inhalation Studies (Diesel Exhaust Plus 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 diesel exhaust up to 12 mg/m^3 (Pepelko and Peirano, 1983).

Heinrich et al. (1986b) exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 4 mg/m^3. See Section 7.3.1.1 for details of the exposure conditions. At the start of exposure the hamsters received either one dose of 4.5 mg diethylnitrosamine (DEN) subcutaneously per kg body weight or 20 weekly intratracheal instillations of 250 µ*g* BaP. Female NMRI mice received weekly intratracheal instillations of 50 or 100 µ*g*BaP for 10 or 20 weeks, respectively, or 50 µ*g* dibenz[ah]anthracene (DBA) for 10 weeks. Additional groups of 96 newborn mice received one s.c. injection of 5 or 10 µ*g* DBA between 24 and 48 hr after birth. Female Wistar rats received weekly subcutaneous injections of dipentylnitrosamine (DPN) at doses of 500 and 250 mg/kg body weight, respectively, during the first 25 weeks of exhaust inhalation exposure. Neither DEN, DBA, or DPN treatment enhanced any tumorigenic responses to diesel exhaust. Response to BaP did not differ from that of BaP alone in hamsters, but results were inconsistent in mice. Although 20 BaP instillations induced a 71% tumor incidence in mice, concomitant diesel exposure resulted in only a 41% incidence. However, neither 10 BaP instillations nor DBA instillations induced significant effects.

Takemoto et al. (1986) exposed Fischer 344 rats for 2 years to diesel exhaust at particle concentrations of 2 to 4 mg/m^3. One month after start of inhalation exposure one group of rats received di-isopropyl-nitrosamine (DIPN) administered i.p. at 1 mg/kg weekly for 3 weeks.

Among injected animals autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were reported in 21 animals exposed to clean air, compared with 12 adenomas and 7 adenocarcinomas in 18 diesel-exposed rats. According to the authors, the incidence of adenocarcinomas was not significantly increased by exposure to diesel exhaust.

Brightwell et al. (1989) investigated the concomitant effects of diesel exhaust and DEN in Syrian hamsters exposed to diesel exhaust diluted to produce particle concentrations of 0.7, 2.2, or 6.6 mg/m^3 for 2 years. The animals received a single dose of 4.5 mg DEN s.c. 3 days prior to start of inhalation exposure. DEN did not affect the lack of responsiveness to diesel exhaust alone. Heinrich et al. (1989b) also exposed Syrian hamsters of both sexes to diesel exhaust diluted to a particle concentration of 3.75 mg/m^3 for up to 18 mo. After 2 weeks of exposure, groups were treated with either 3 or 6 mg DEN/kg body weight, respectively. Again, DEN did not significantly influence the lack of tumorigenic responses to diesel exhaust.

Heinrich et al. (1989a) investigated the effects of DPN in female Wistar rats exposed to diesel exhaust diluted to achieve a particle concentration of 4.24 mg/m^3 for 2-2.5 years. DPN at doses of 250 and 500 mg/kg body weight was injected subcutaneously once a week for the first 25 weeks of exposure. The tumorigenic responses to DPN were not affected by exposure to diesel exhaust. For details of exposure conditions of the hamster studies see Section 7.3.1.3.

Heinrich et al. (1986a) and Mohr et al. (1986) compared the effects of exposure to particles having only a minimal carbon core but a much greater concentration of PAHs than DPM does. The desired exposure conditions were achieved by mixing coal oven flue gas with pyrolyzed pitch. The concentration of B[a]P and other PAHs per milligram of DPM was about three orders of magnitude greater than that of diesel exhaust. Female rats were exposed to the flue gas-pyrolyzed pitch for 16 hr/day, 5 days/week at particle concentrations of 3 to 7 mg/m^3 for 22 mo, then held in clean air for up to an additional 12 mo. Among 116 animals exposed, 22 tumors were reported in 21 animals, for an incidence of 18.1%. One was a bronchioloalveolar adenoma, one was a bronchioloalveolar carcinoma, and 20 were squamous cell tumors. Among the latter, 16 were classified as benign keratinizing cystic tumors and 4 were classified as carcinomas. No tumors were reported in 115 controls. The tumor incidence in this study was comparable to that reported previously for the diesel exhaust-exposed animals.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole diesel exhaust, coal oven flue gas, or carbon black (15.8%, 18.1%, and 8% to 17%, respectively) was very similar. This occurred despite the fact that the PAH content of the PAH-enriched pyrolyzed pitch was more than three orders of magnitude greater than that of diesel exhaust; carbon black, on the other hand, had only traces of PAHs. Based on these

findings, particle-associated effects appear to be the primary cause of diesel-exhaust-induced lung cancer in rats exposed at high concentrations. This issue is discussed further in Chapter 7.

7.3.4. Lung Implantation or Intratracheal Instillation Studies

7.3.4.1. *Rat Studies*

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that PAHs in diesel exhaust that consist of four or more rings have carcinogenic potential. Condensate was obtained from the whole exhaust of a 3.0-L passenger-car diesel engine connected to a dynamometer operated under simulated city traffic driving conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25% and 75% of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were surgically implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (B[a]P) controls were also included in the protocol (Table 7-6). Fraction Ilb (made up of PAHs with four to seven rings), which accounted for only 0.8% of the total weight of DPM condensate, produced the highest incidence of carcinomas following implantation into rat lungs. A carcinoma incidence of 17.1% was observed following implantation of 0.21 mg IIb/rat, whereas the nitro-PAH fraction (IId) at 0.18 mg/rat accounted for only a 2.8% carcinoma incidence. Hydrophilic fractions of the DPM extracts, vehicle (beeswax/trioctanoin) controls, and untreated controls failed to exhibit carcinoma formation. Administration of all hydrophobic fractions (IIa-d) produced a carcinoma incidence (20%) similar to the summed incidence of fraction IIb (17.1%) and IId (2.8%). The B[a]P positive controls (0.03, 0.1, 0.3 mg/rat) yielded a carcinoma incidence of 8.6%, 31.4%, and 77.1%, respectively. The study showed that the tumorigenic agents were primarily four- to seven-ring PAHs and, to a lesser extent, nitroaromatics. However, these studies demonstrated that simultaneous administration of various PAH compounds resulted in a varying of the tumorigenic effect, thereby implying that the tumorigenic potency of PAH mixtures may not depend on any one individual PAH. This study did not provide any information regarding the bioavailability of the particle-associated PAHs that might be responsible for carcinogenicity.

Kawabata et al. (1986) compared the effects of activated carbon and diesel exhaust on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with DPM (1 mg/week for 10 weeks). A second group of 31 rats was instilled with activated carbon using the same dosing regime. Twenty-seven rats received only the solvent (buffered saline with 0.05% Tween 80), and 53 rats were uninjected. Rats dying after 18 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 diesel exhaust particles, compared with 2.1% in rats instilled with 15 mg particles extracted to remove all organic constituents, and none among controls. Instillation of 30 mg of extracted particles induced a 14.6% incidence of squamous lesions, indicating the greater effectiveness of particles alone as lung particle overload increased.

Iwai et al. (1997) instilled 2, 4, 8, and 10 mg of whole diesel particles over a 2- to 10-week period into female F/344 rats, 50 or more per group. Tumors were reported in 6%, 20%, 43%, and 74% of the rats, with incidence of malignant tumors equal to 2%, 13%, 34%, and 48%, respectively. In a second experiment comparing whole with extracted diesel particles, tumor incidence equaled 1/48 (2%) in uninjected controls, 3/55 (5%) in solvent controls, 12/56 (21%) in extracted diesel particles, and 13/106 (12%) in animals injected with unextracted particles. Although the extracted particles appeared to be more potent, when converted to a lung burden basis (mg/100 mg dry lung) the incidence was only 14% among those exposed to extracted exhaust compared with 31% in those exposed to whole particles.

Dasenbrock et al. (1996) conducted a study to determine the relative importance of the organic constituents of diesel particles and particle surface area in the induction of lung cancer in rats. Fifty-two female Wistar rats were intratracheally instilled with 16-17 doses of DPM, extracted DPM, printex carbon black (PR), lampblack (LB), benzo[*a*]pyrene (B*a*P), DPM + B*a*P, or PR + B*a*P. The animals were held for a lifetime or sacrificed when moribund. The lungs were necropsied and examined for tumors. Diesel particles were collected from a Volkswagen 1.6-L engine operating on a US FTP-72 driving cycle. The mass median aerodynamic diameter (MMAD) of the diesel particles was 0.25 µm and the specific surface area was 12 m^2/gm. Following extraction with toluene, specific surface area increased to 138 m^2/gm. The MMAD for extracted PR was equal to 14 nm, while the specific surface area equaled 271 m^2/gm. The MMAD for extracted lampblack was equal to 95 nm, with a specific surface area equal to 20 m^2/gm. The B*a*P content of the treated particles was 11.3 mg per gm diesel particles and 29.5 mg

B*a*P per gm PR. Significant increases in lung tumors were detected in rats instilled with 15 mg unextracted DPM and 30 mg extracted DPM, but not 15 mg extracted DPM. Printex CB was more potent than lampblack CB for induction of lung tumors, 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 diesel exhaust may be of greater importance in the induction of lung tumors at low doses pf B*a*P (0.2-0.4 mg).

7.3.4.2. *Syrian Hamster Studies*

Kunitake et al. (1986) and Ishinishi et al. (1988b) conducted a study in which total doses of 1.5, 7.5, or 15 mg of a dichloromethane extract of DPM were instilled intratracheally over 15 weeks into male Syrian hamsters that were then held for their lifetimes. The tumor incidences of 2.3% (1/44), 0% (0/56), and 1.7% (1/59) for the high-, medium-, and low-dose groups, respectively, did not differ significantly from the 1.7% (1/56) reported for controls. Addition of 7.5 mg of B[a]P to a DPM extract dose of 1.5 mg resulted in a total tumor incidence of 91.2% and malignant tumor incidence of 88%. B[a]P (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2% (85% of these being malignant), which was not significantly different from the DPM extract + B[a]P group. Intratracheal administration of 0.03 µg B[a]P, the equivalent content in 15 mg of DPM extract, failed to cause a significant increase in tumors in rats. This study demonstrated a lack of detectable interaction between DPM extract and B[a]P, the failure of DPM extract to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of high doses of B[a]P. For studies using the DPM extract, some concern must be registered regarding the known differences in chemical composition between DPM extract and DPM. As with all intratracheal instillation protocols, DPM extract lacks the complement of volatile chemicals found in whole diesel exhaust.

The effects on hamsters of intratracheally instilled DPM suspension, DPM with Fe_2O_3, or DPM extract with Fe_2O_3 as the carrier were studied by Shefner et al. (1982). The DPM component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0

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 diesel exhaust, the relevant mode for human exposure, had been reported. Since then long-term inhalation bioassays with diesel exhaust have been carried out in the United States, Germany, Switzerland, and Japan, testing responses of rats, mice, and Syrian hamsters, and to a limited extent cats and monkeys.

It can be reasonably concluded that with adequate exposure, inhalation of diesel exhaust is capable of inducing lung cancer in rats. Responses best fit cumulative exposure (concentration × daily exposure duration × days of exposure). Examination of rat data shown in Table 7-8 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 diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986b; Stöber, 1986) and in female Sencar mice (Pepelko and Peirano, 1983). An apparent increase was also seen in female C57BL mice (Takemoto et al., 1986). However, in a repeat of their earlier study, Heinrich et al. (1995) failed to detect lung tumor induction in either NMRI or C57BL/6N mice. No increases in lung tumor rates were reported in a series of inhalation studies using strain A mice (Orthoefer et al., 1981; Kaplan et al., 1982, 1983; White et al., 1983). Finally, Mauderly et al. (1996) reported no tumorigenic responses in CD-1 mice exposed under conditions resulting in positive responses in rats. The successful induction of lung tumors in mice by Ichinose et al. (1997a,b) via intratracheal instillation may have been the result of focal deposition of larger doses. Positive effects in Sencar mice may be due to use of a strain sensitive to tumor induction in epidermal tissue by organic agents, as well as exposure from conception, although proof for such a hypothesis is lacking.

Attempts to induce significant increases in lung tumors in Syrian hamsters by inhalation of whole diesel exhaust were unsuccessful (Heinrich et al., 1982, 1986b, 1989b; Brightwell et al., 1986). However, hamsters are considered to be relatively insensitive to lung tumor induction. For example, while cigarette smoke, a known human carcinogen, was shown to induce laryngeal cancer in hamsters, the lungs were relatively unaffected (Dontenwill et al., 1973).

Neither cats (Pepelko and Peirano, 1983 [see Chapter 7]) nor monkeys (Lewis et al., 1986) developed tumors following 2-year exposure to diesel exhaust. The duration of these exposures, however, was likely to be inadequate for these two longer-lived species, and group sizes were quite small. Exposure levels were also below the maximum tolerated dose (MTD) in the monkey studies and, in fact, only borderline for detection of lung tumor increases in rats.

Long-term exposure to diesel exhaust filtered to remove particulate matter failed to induce lung tumors in rats (Heinrich et al., 1986b; Iwai et al., 1986; Brightwell et al., 1989), or in Syrian hamsters (Heinrich et al., 1986b; Brightwell, 1989). A significant increase in lung carcinomas was reported by Heinrich et al. (1986b) in NMRI mice exposed to filtered exhaust. However, in a more recent study the authors were unable to confirm earlier results in either NMRI or C57BL/6N mice (Heinrich et al., 1995). Although filtered exhaust appeared to potentiate the carcinogenic effects of DEN (Heinrich et al., 1982), because of the lack of positive data in rats and equivocal or negative data in mice it can be concluded that filtered exhaust is either not carcinogenic or has a low cancer potency.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of DPM. Rittinghausen et al. (1997) reported an increase in cystic keratinizing epitheliomas following intratracheal instillation of rats with either original DPM or DPM extracted to remove the organic fraction, with the unextracted particles inducing a slightly greater effect. Grimmer et al. (1987) showed not only that an extract of DPM was carcinogenic when instilled in the lungs of rats, but also that most of the carcinogenicity resided in the portion containing PAHs with four to seven rings. Intratracheal instillation did not induce lung tumors in Syrian hamsters (Kunitake et al., 1986; Ishinishi et al., 1988b).

Dermal exposure and s.c. injection in mice provided additional evidence for tumorigenic effects of DPM. Particle extracts applied dermally to mice have been shown to induce significant skin tumor increases in two studies (Kotin et al., 1955; Nesnow et al., 1982). Kunitake et al. (1986) also reported a marginally significant increase in skin papillomas in ICR mice treated with an organic extract from an HD diesel engine. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of DPM suspensions. However, in this study the exhaust particles were collected at temperatures of 100 °C, which would minimize the condensation of vapor-phase organics and, therefore, reduce the availability of potentially carcinogenic compounds that might normally be present on diesel exhaust particles. A significant increase in the incidence of sarcomas in female C57Bl mice was reported by Kunitake et al. (1986) following s.c. administration of LD DPM extract at doses of 500 mg/kg. Takemoto et al. (1988) provided additional data for this study and reported an increased tumor incidence in the mice following injection of LD engine DPM extract at doses of 100 and 500 mg/kg. Results of i.p. injection of DPM or DPM extracts in strain A mice were generally negative (Orthoefer et al., 1981; Pepelko and Peirano, 1983), suggesting that the strain A mouse may not be a good model for testing diesel emissions.

Results of experiments using tumor initiators such as DEN, B[a]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986b; Takemoto et al., 1986) were generally inconclusive regarding the tumor-promoting potential of either filtered or whole diesel exhaust. A

report by Heinrich et al. (1982), however, indicated that filtered exhaust may promote the tumor-initiating effects of DEN in hamsters.

Several reports (Wong et al., 1985; Bond et al., 1990) affirm observations of the potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. These findings are discussed in more detail in Section 3.6. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 4.

Evidence for the importance of the carbon core was initially provided by studies of Kawabata et al. (1986), which showed induction of lung tumors following intratracheal instillation of carbon black that contained no more than traces of organics, and studies of Heinrich (1990) that indicated that exposure via inhalation to carbon black (Printex 90) particles induced lung tumors at concentrations similar to those effective in DPM studies. Additional studies by Heinrich et al. (1995) and Nikula et al. (1995) confirmed the capability of carbon particles to induce lung tumors. Induction of lung tumors by other particles of low solubility, such as titanium dioxide (Lee et al., 1986), confirmed the capability of particles to induce lung tumors. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having much higher PAH concentrations than DPM, also was effective in tumor induction (Heinrich et al., 1986a, 1994).

The relative importance of the adsorbed organics, however, remains to be elucidated and is of some concern because of the known carcinogenic capacity of some of these chemicals. These include polycyclic aromatics as well as nitroaromatics, as described in Chapter 2. Organic extracts of particles also have been shown to induce tumors in a variety of injection, intratracheal instillation, and skin-painting studies, and Grimmer et al. (1987) have, in fact, shown that the great majority of the carcinogenic potential following instillation resided in the fraction containing four- to seven-ring PAHs.

In summary, based on positive inhalation 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 diesel exhaust is considered to be adequate in animals. The contribution of the various fractions of diesel exhaust to the carcinogenic response is less certain. Exposure to filtered exhaust generally failed to induce lung tumors. The presence of known carcinogens adsorbed to diesel particles and the demonstrated tumorigenicity of particle extracts in a variety of injection, instillation, and skin-painting studies indicate a carcinogenic potential for the organic fraction. Studies showing that 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 diesel exhaust to induce lung tumors at non-particle-

overload conditions, and the relative contribution of the particles' core versus the particle-associated organics (if effects do occur at low doses) remains to be determined.

7.4. MODE OF ACTION OF DIESEL EMISSION-INDUCED CARCINOGENESIS

As noted in Chapter 2, diesel exhaust 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., 1986), discussion will focus on the particulate matter phase. Additive or synergistic effects of vapor-phase components, however, cannot be totally discounted, as chronic inhalation bioassays involving exposure to diesel particles alone have not been carried out.

Several hypotheses regarding the primary mode of action of diesel exhaust have been proposed. Initially it was generally believed that cancer was induced by particle-associated organics acting via a genotoxic mechanism. By the late 1980s, however, studies indicated that carbon particles virtually devoid of organics could also induce lung cancer at sufficient inhaled concentrations (Heinrich, 1990). This finding provided support for a hypothesis originally proposed by Vostal (1986) that induction of lung tumors arising in rats exposed to high concentrations of diesel exhaust is related to overloading of normal lung clearance mechanisms, accumulation of particles, and cell damage followed by regenerative cell proliferation. The action of particles is therefore mediated by epigenetic mechanisms that can be characterized more by promotional than initiation stages of the carcinogenic process. More recently several studies have focused upon the production of reactive oxygen species generated from particle-associated organics, which may induce oxidative DNA damage at exposure concentrations lower than those required to produce lung particle overload. 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 diesel exhaust are benzo(*a*)pyrene (B[*a*]P), dibenz[*a*,*h*]anthracene, pyrene, chrysene, and nitroarenes such as 1-NP, 1,3-DNP, 1,6-DNP, and 1,8-DNP, all of which are mutagenic, carcinogenic, or implicated as procarcinogens or cocarcinogens (Stenback et al., 1976; Weinstein and Troll, 1977; Thyssen et al., 1981; Pott and Stöber, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988). More recently Enya et al. (1997) reported isolation of 3-nitrobenzanthrone, one of the most powerful direct-acting mutagens known to date, from the organic extracts of diesel exhaust.

Grimmer et al. (1987) separated diesel exhaust particle extract into a water- and a lipid-soluble fraction, and the latter was further separated into a PAH-free, a PAH-containing, and a polar fraction by column chromatography. These fractions were then tested in Osborne-Mendel rats by pulmonary implantation at doses corresponding to the composition of the original diesel exhaust. The water-soluble fraction did not induce tumors; the incidences induced by the lipid-soluble fractions were 0% with the PAH-free fraction, 25% with the PAH and nitro-PAH-containing fractions, and 0% with the polar fraction. The PAH and nitro-PAH-containing fraction, comprising only 1% by weight of the total extract, was thus shown to be responsible for most, if not all, of the carcinogenic activity.

Exposure of rats by inhalation to 2.6 mg/m^3 of an aerosol of tar-pitch condensate with no carbon core but containing 50 µg/m^3 benzo[*a*]pyrene along with other PAHs for 10 months induced lung tumors in 39% of the animals. The same amount of tar-pitch vapor condensed onto the surface of carbon black particles at 2 and 6 mg/m^3 resulted in tumor rates that were roughly two times higher (89% and 72%). 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 benzo[*a*]pyrene content in the coal-tar pitch was about three orders of magnitude greater than in diesel soot. Moreover, because organics are present on diesel particles in a thinner layer and the particles are quite convoluted, they may be more tightly bound and less bioavailable. Nevertheless, these studies provide evidence supporting the involvement of organic constituents of diesel particles in the carcinogenic process.

Exposure of humans to related combustion emissions provides some evidence for the involvement of organic components. Mumford et al. (1989) reported greatly increased human lung cancer mortality in Chinese communes burning so-called smoky coal, but not wood, in unvented open-pit fires used for heating and cooking. Although particle concentrations were similar, PAH levels were five to six times greater in the air of communes burning smoky coal. Coke oven emissions, containing high concentrations of PAHs but lacking an insoluble carbon core, have also been shown to be carcinogenic in humans (Lloyd, 1971).

Adsorption of PAHs to a carrier particle such as hematite, CB, aluminum, or titanium dioxide enhances their carcinogenic potency (Farrell and Davis, 1974). As already noted, adsorption to carbon particles greatly enhanced the tumorigenicity of pyrolyzed pitch condensate containing B[*a*]P and other aromatic carcinogens (Heinrich et al., 1995). The increased effectiveness can be partly explained by more efficient transport to the deep lung. Slow release also enhances residence time in the lungs and prevents overwhelming of activating pathways. As discussed in Chapter 3, free organics are likely to be rapidly absorbed into the bloodstream, which may explain why the vapor-phase component of exhaust is relatively ineffective in the induction of pathologic or carcinogenic effects.

Even though the organic constituents may be tightly bound to the particle surface, significant elution is still likely because particle clearance half-times are nearly 1 year in humans (Bohning et al., 1982). Furthermore, Gerde et al. (1991a) presented a model demonstrating that large aggregates of inert dust containing crystalline PAHs are unlikely to form at doses typical of human exposure. This allows the particles to deposit and react with the surrounding lung medium, without interference from other particles. Particle-associated PAHs can then be expected to be released more rapidly from the particles. Bond et al. (1984) provided evidence that alveolar macrophages from beagle dogs metabolized B[*a*]P coated on diesel particles to proximate carcinogenic forms. Unless present on the particle surface, B[*a*]P is more likely to pass directly into the bloodstream and escape activation by phagocytic cells.

The importance of DE-associated PAHs in the induction of lung cancer in humans may be enhanced because of the possibility that the human lung is more sensitive to these compounds than are rat lungs. Rosenkranz (1996) summarized information indicating that in humans and mice, large proportions of lung cancers contain both mutated *p*53 suppressor genes and K-*ras* genes. Induction of mutations in these genes by genotoxins, however, is much lower in rats than in humans or mice.

B[*a*]P, although only one of many PAHs present in diesel exhaust, is the one most extensively studied. Bond et al. (1983, 1984) demonstrated metabolism of particle-associated B[*a*]P and free B[*a*]P by alveolar macrophages (AM) and by type II alveolar cells. The respiratory tract cytochrome P-450 systems have an even greater concentration in the nonciliated

bronchiolar cells (Boyd, 1984). It is 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 benzo[*a*] pyrene; 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 diesel exhaust 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 diesel exhaust genotoxic effect.

Nitro-PAHs have also been implicated as potentially involved in diesel-exhaust-induced lung cancer. Although the nitro-PAH fraction of diesel was less effective than PAHs in the induction of lung cancer when implanted into the lungs of rats (Grimmer et al., 1987), in a study of various extracts of diesel exhaust particles, 30%-40% of the total mutagenicity could be attributed to a group of six nitroarenes (Salmeen et al., 1984). Moreover, Gallagher et al. (1994) reported results suggesting that DNA adducts are formed from nitro-PAHs present in DNA and may play a role in the carcinogenic process. Nitroarenes, however, quantitatively represent a very small percentage of diesel particle extract (Grimmer et al., 1987), making their role in the tumorigenic response uncertain.

The induction of DNA adducts in humans occupationally exposed to diesel exhaust indicates the likelihood that PAHs are participating in the tumorigenic response, and that these effects can occur at exposure levels less than those required to induce lung particle overload. Distinct adduct patterns were found among garage workers occupationally exposed to diesel exhaust when compared 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 diesel exhaust exposure compared with unexposed mechanics. Hou et al. (1995) found elevated adduct levels in bus maintenance workers exposed to diesel exhaust. Although no difference in

mutant frequency was observed between the groups, the adduct levels were significantly different (3.2 vs. 2.3×10^{-8}). Nielsen et al. (1996) measured three biomarkers in DE-exposed bus garage workers: lymphocyte DNA adducts, hydroxyethylvaline adducts in hemoglobin, and 1-hydroxypyrene in urine. Significantly increased levels were reported for all three. Qu et al. (1996) detected increased adduct levels, as well as increases in some individual adducts, in the blood of underground coal miners exposed to DE.

7.4.2. Role of Inflammatory Cytokines and Proteolytic Enzymes in the Induction of Lung Cancer 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 diesel exhaust.

7.4.4. Relationship of Physical Characteristics of Particles to Cancer Induction

The biological potential of inhaled particles is strongly influenced by surface chemistry and character. For example, the presence of trace metal compounds such as aluminum and iron, as well as ionized or protonated sites, is important in this regard (Langer and Nolan, 1994). A major factor is specific surface area (surface area/mg). PMNs characteristically are increased abnormally in the lung by diesel exhaust exposure, but their presence in the lungs does not appear to be excessive following the pulmonary deposition of even high lung burdens of 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 paticle 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 diesel exhaust, carbon black, or titanium dioxide, irrespective of particle type. Langer and Nolan (1994) reported that the hemolytic potential of Min-U-Sil15, a silica flour, increased in direct relationship to specific surface area at nominal particle diameters ranging from 0.5 to 20 μm.

Ultrafine particles appear to be more likely to be taken up by lung epithelial cells. Riebe-Imre et al. (1994) reported that CB is taken up by lung epithelial cells in vitro, inducing chromosomal damage and disruption of the cytoskeleton, lesions that closely resemble those present in tumor cells. Johnson et al. (1993) reported that 20-nm polytetrafluoroethylene particles are taken up by pulmonary epithelial cells as well as polymorphonuclear leucocytes, inducing an approximate 4-, 8-, and 40-fold increase in the release of interleukin-1 alpha and beta, inducible nitric oxide synthetase, and macrophage inflammatory protein, respectively.

The carcinogenic potency of diesel particles, therefore, appears to be related, at least to some extent, to their small size and convoluted shape, which results in a large specific particle surface area. Toxicity and carcinogenicity increased with increasing 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 diesel exhaust 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 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 diesel exhaust 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 concentrations, inflammatory effects associated with lung particle overload are generally absent. However, activation of organic carcinogens and generation of oxidants from the organic fraction can still be expected. Actual contribution depends upon elution and the effectiveness of antioxidants. Direct effects of ultrafine diesel particles taken up by epithelial cells are also likely to play a role.

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 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.

7.4.6. Summary

Recent studies have shown tumor rates resulting from exposures to nearly organic-free CB particles at high concentrations to be similar to those observed for diesel exhaust exposures, thus providing strong evidence for a particle overload mechanism for DE-induced pulmonary carcinogenesis in rats. Such a mechanism is also supported by the fact that carbon particles per se cause inflammatory responses and increased epithelial cell proliferation and that AM function may be compromised under conditions of particle overload.

The particle overload hypothesis appears sufficient to account for DE-induced lung cancer in rats. However, there is increasing evidence for lung cancer induction in humans at concentrations insufficient to induce lung particle overload as seen in rats (Section 3.7 and ILSI 2000). Uptake of particles by epithelial cells at ambient or occupational exposure levels, DNA damage resulting from oxygen-free radicals generated from organic molecules, and the gradual in

situ extraction and activation of procarcinogens associated with the diesel particles are likely to play a role in this response. The slower particle clearance rates in humans (up to a year or more) may result in greater extraction of organics. This is supported by reports of increased DNA adducts in humans occupationally exposed to diesel exhaust at concentrations unlikely to induce lung particle overload. 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 diesel exhaust and other particles at high exposure concentrations may be species-specific and not particle-specific. The likelihood that different modes of action predominate at high and low doses also renders low-dose extrapolation to ambient concentrations uncertain.

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

A 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. Accordingly, the question to be addressed in hazard characterization is expanded to how likely an agent is to be a human carcinogen, and under what exposure conditions a cancer hazard may be expressed. 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.

To express the weight of evidence for potential human carcinogenicity, EPA's proposed guidelines utilize a hazard narrative in place of the classification system. However, in order to provide some measure of consistency, standard hazard descriptors are used as part of the hazard narrative to express the conclusion regarding the weight of evidence for potential human carcinogenicity.

The sections to follow evaluate and weigh the individual lines of evidence and combine all evidence to make an informed judgement about the potential human carcinogenicity 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 Revised 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 these 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, confounding by smoking is unlikely in these studies because the comparison population was 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 Chapter 7 (Section 7.2.4.5), application of the criteria for causality provides evidence 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 of workers to DE) and an inability to satisfactorily account for all 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 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 mixed results whereas negative findings were consistently seen in hamsters. The gaseous phase of DE (filtered exhaust without particulate fraction), however, was found not to be carcinogenic in rats, mice, or hamsters.

In several intratracheal instillation studies, diesel particulate matter (DPM), DPM extracts, and carbon black, which was 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.

Overall, there is sufficient evidence for the potential carcinogenicity of whole DE in the rat at high exposure concentration or administered dose, both by inhalation and intratracheal instillation. Available data indicate 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 gaseous phase of DE, however, does not appear to have any significant role in DE-induced lung cancer response in the rat.

Available data also indicate that among the traditional animal test species, the rat is the most sensitive species to DE. As reviewed in Section 7.4, the lung cancer responses in rats from high-concentration exposures to DE 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 animal evidence (i.e., increased lung tumors in the rat) provides additional support for identifying

potential cancer hazard to humans, but is not considered suitable for dose-response analysis and estimation of human risk to DE.

The consistent findings of carcinogenic activity by the organic extracts of DPM in noninhalation studies (intratracheal instillation, lung implantation, skin painting) further contribute to the overall animal evidence for a human hazard potential for DE.

7.5.3. Other Key Data

Other key data, although not as extensive as the human and animal carcinogenicity 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). 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 (Section 3.6).

7.5.4. Mode of Action

As discussed in Section 7.4, the modes of action of DE-induced carcinogenicity in humans are not well understood. It is likely that multiple modes of action are 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 appears to have the greatest 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 also appears to be related to the small size of the particles. Moreover, the relative contribution of the various modes of action may be different at different exposure levels. Available evidence from animal studies indicates the importance of the role of the DE particles 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 Carcinogen Risk Assessment Guidelines

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

- Limited human evidence (less than sufficient) for a causal association between DE exposure and increased lung cancer risk among workers of different occupations;
- Sufficient animal evidence for the induction of lung cancer in the rat from inhalation exposure to high concentrations of DE, DPM, and the carbon core; and supporting evidence of carcinogenicity of DPM and the associated organics in rats and mice by noninhalation route of exposure; 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 the known mutagenic and/or carcinogenic activity of a number of individual organic compounds present on the particles and in the gaseous phase.

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

The combined evidence supports the conclusion that DE is *likely to be carcinogenic to humans* by inhalation exposure at any exposure condition. In comparison with other agents designated as likely to be carcinogenic to humans, the weight of evidence for DE is at the upper end of the spectrum. The weight of evidence of human carcinogenicity is based on:

- Strong but less than sufficient epidemiologic evidence for a causal association between occupational exposure and elevated risk of lung cancer;
- Consistent evidence of increases of lung tumors in rats from chronic inhalation exposure to high concentration of whole DE, DPM, or the particle elemental carbon core;
- Supportive evidence of carcinogenicity in rats for the diesel particle (DPM) via intratracheal instillation, and for DPM organic extracts in rats and mice in noninhalation studies (intratracheal instillation, lung implantation, skin painting, subcutaneous injection);

- Extensive evidence of mutagenic and chromosomal effects of DE and its organic constituents;
- Suggestive evidence of the bioavailability of the DPM organics in studies of humans and animals; and
- The presence of a number of individual organic compounds on the diesel particles (e.g., PAHs and derivatives) and in the gaseous phase (e.g., benzene, acetaldehydes) that are known to exhibit mutagenic and/or carcinogenic properties.

A major uncertainty in characterizing the potential cancer hazard for DE at low levels of environmental exposure is the incomplete understanding of its mode of action for the induction of lung cancer in humans. Nonetheless, available data indicate that DE-induced lung carcinogenicity seems to be mediated by mutagenic and nonmutagenic events by 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 any exposure level. 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. Accordingly, linear low-dose extrapolation should be assumed in dose-response assessment. 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 cancer hazard to humans. 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, although a role for the gas-phase components cannot be ruled out. Using either EPA's 1986 Carcinogen Risk Assessment Guidelines (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. Many experts view the evidence as weak while many others consider the evidence as strong. This is due to a lack of consensus about whether the effects of smoking have been adequately accounted for in key studies, and the lack of historical DE exposure data for the available studies.
- Although the mode of action for DE-induced lung tumors in rats from high exposure is sufficiently understood, the mode of action for lung cancer risk in humans is not fully known. To date, available evidence for the role of both the adsorbed organics and the carbon core particle has been shown to be associated with high exposure conditions. There is virtually no information about the relative role of DE constituents in mediating carcinogenic effects at the low exposure levels. Furthermore, there is only a limited understanding regarding the relationship between 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 clearly identified.

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

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

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Waller (1981)	Approximately 20,000 male London transportation workers Aged 45 to 64 years 25 years follow-up (1950-1974)	Five job categories used to define exposure Environmental benzo[a]pyrene concentrations measured in 1957 and 1979	SMR = 79 for lung cancer for the total cohort SMRs for all five job categories were less than 100 for lung cancer	Exposure measurement of benzo[a]pyrene showed very little difference between inside and outside the garage Incomplete information on cohort members No adjustment for confounding such as other exposures, cigarette smoking, etc. No latency analysis
Howe et al. (1983)	43,826 male pensioners of the Canadian National Railway Company Mortality between 1965 and 1977 among these pensioners was compared with mortality of general Canadian population	Exposure groups classified by a group of experts based on occupation at the time of retirement Three exposure groups: Nonexposed Possibly exposed Probably exposed	RR = 1.2 (p=0.013) and RR = 1.3 (p=0.001) for lung cancer for possible and probable exposure, respectively A highly significant dose-response relationship demonstrated by trend test (p<0.001)	Incomplete exposure assessment due to lack of lifetime occupational history Mixed exposures to coal dust/combustion products and diesel exhaust 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 diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust 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 diesel exhaust exposure	SMR = 133 (p<0.03) for lung cancer in the general hand job group Several other job categories showed SS increased SMRs for several other sites based on fewer than five cases	Ill-defined diesel exhaust exposure without any ranking Average 6-year follow-up i.e., not enough time for lung cancer latency No adjustment for confounders
Wong et al. (1985)	34,156 male heavy construction equipment operators Members of the local union for at least 1 year between January 1, 1964, and December 1, 1978	20 functional job titles grouped into three job categories for potential exposure Exposure groups (high, low, and unknown) based on job description and proximity to source of diesel exhaust emissions	SMR = 166 (p<0.05) for liver cancer for total cohort SMR = 343 (observed = 5, p<0.05) for lung cancer for high-exposure bulldozer operators with 15-19 years of membership, 20+ years of follow-up SMR = 119 (observed = 141, p<0.01) for workers with no work histories	No validation of exposure categories, which were based on surrogate information Incomplete employment records Employment history other than from the union not available 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 diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust 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 diesel exhaust 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

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

Authors	Population studied	Diesel exhaust exposure assessment	Results	Limitations
Garshick et al. (1988)	55,407 white male railroad workers Aged 40 to 64 years in 1959 Started work 10-20 years earlier than 1959	Industrial hygiene data correlated with job titles to dichotomize the jobs as "exposed" or "not exposed"	RR = 1.45 (40-44 year age group) RR = 1.33 (45-49 year age group) Both SS After exclusion of workers exposed to asbestos RR = 1.57 (40-44 year age group) RR = 1.34 (45-49 year age group) Both SS	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 diesel exhaust versus those who were not
Garshick (1991)			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 et al. (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 diesel exhaust: cohort mortality studies (continued)

Authors	Population studied	Diesel exhaust 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 diesel exhaust 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	Diesel exhaust 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	Diesel exhaust 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

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

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

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

Authors	Population studied	Diesel exhaust exposure	Results	Limitations
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 diesel exhaust exposure No validation of exposure done Underground miners data not adjusted for other confounders such as radon, etc.

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

Authors	Population studied	Diesel exhaust exposure	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 diesel exhaust exposure	Exposure based on occupational history and self-report, which was not validated 50% occupational history provided by next of kin Absence of lung cancer association with asbestos suggests misclassification of exposure
Garshick et al. (1987)	1,319 lung cancer cases who died between March 1, 1981, and February 28, 1982 2,385 matched controls (two each, age and date of death) Both cases and controls drawn from railroad worker cohort who had worked for 10 or more years	Personal exposure assessed for 39 job categories This was corrected with job titles to dichotomize the exposure into: Exposed Not exposed Industrial hygiene sampling done	SS OR = 1.41 (≤64 year age group) SS OR = 1.64 (≤64 year age group) for ≥20 years diesel exhaust exposure group when compared to 0- to 4-year exposure group All ORs adjusted for lifetime smoking and asbestos exposure	Probable misclassification of diesel exhaust exposure jobs Years of exposure used as surrogate for dose 13% of death certificates not ascertained Overestimation of smoking history
Benhamou et al. (1988)	1,260 histologically confirmed lung cancer cases 2,084 non-tobacco-related disease matched controls (sex, age at diagnosis, hospital admission, and interviewer) Occurring between 1976 and 1980 in France	Based on exposures determined by panel of experts The occupations were recorded blindly using International Standard Classification of Occupations as chemical or physical exposures	Significant excess risks were found in motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35) (smoking adjusted)	Exposure based on occupational histories not validated Exposures classified as chemical and physical exposures, not specific to diesel exhaust

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

Authors	Population studied	Diesel exhaust exposure	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 diesel exhaust exposure) Lowest diesel exhaust 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 diesel exhaust: case-control studies of lung cancer (continued)

Authors	Population studied	Diesel exhaust exposure	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 diesel exhaust	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 diesel exhaust was small
Emmelin et al. (1993)	50 male lung cancer cases from 15 ports (worked for at least 6 months between 1950 and 1974), 154 controls matched on age and port	Indirect diesel exhaust exposure assessment done based on (1) exposure intensity, (2) characteristics of ventilation, (3) measure of proportion of time in higher exposure jobs	SS OR for high-exposure group = 6.8 Positive trend for diesel exhaust 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

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

Authors	Population studied	Diesel exhaust exposure	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 diesel exhaust	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 diesel exhaust: case-control studies of lung cancer (continued)

Authors	Population studied	Diesel exhaust exposure	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 diesel exhaust 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 diesel exhaust 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.

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) / 0/96 (0) / 0/96 (0) / 0/96 (0) 0/92 (0) / 0/92 (0) / 0/92 (0) / 0/92 (0) 8/95 (8.4) / 0/95 (0) / 9/95 (9.4) / 17/95 (17.8)[c]	
								Adenomas / Adenocarcinoma and adenosquamous carcinoma / Large cell and squamous cell carcinomas / All tumors	
Iwai et al. (1986)	Rat/F344	F, 24 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) / 0/22 (0) / 0/22 (0) / 1/22 (4.5)[f] 0/16 (0) / 0/16 (0) / 0/16 (0) / 0/16 (0) 3/19 (0) / 3/19 (15.8) / 2/19 (10.5) / 8/19 (42.1)[c,g]	
								Adenoma / Carcinoma	
Takemoto et al. (1986)	Rat/F344	F, 12 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) / 0/12 (0) 10/21 (47.6) / 4/21 (19) 0/15 (0) / 0/15 (0) 12/18 (66.7) / 7/18 (38.9)	
								Adenomas / Adenocarcinoma + squamous cell carcinoma / Squamous cysts / All tumors	
Mauderly et al. (1987)	Rat/F344	M + F, 230[b] 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) / (0.9) / (0) / (0.9) (0) / (1.3) / (0) / (1.3) (2.3) / (0.5) / (0.9) / (3.6)[c] (0.4) / (7.5) / (4.9) / (12.8)[c]	

Study	Species/	Sex/total number	atmosphere	Particle concentration (^{3})	treatment	Exposure	Post-exposure	Tumor type and incidence (%)[a]				
								Adenomas	Adenosquamous carcinomas	Squamous carcinomas	All tumors	
Ishinishi et al.	Rat/F344		Clean air	0		16 hr/day,		0/123 (0)			1/123 (0.8)	
(1988a)		M + F, 123		0.5	None				0/123 (0)	0/123 (0)		
		M + F, 125	Whole exhaust		None	for up to		0/125 (0)	0/125 (0)		0/125 (0)	
			Whole exhaust	1.8		30 mo		0/123 (0)		0/125 (0)	4/123 (3.3)	
engine		M + F, 124		3.7	None				6/124 (4.8)	0/123 (0)	[c]	
								Adenomas		All tumors		
Ishinishi et al.	Rat/F344											
		NS, 8	Whole exhaust	0.1	None	6 days/week,	12 mo	0/8 (0)	0/8 (0)	0/8 (0)		
		NS, 11	Whole exhaust	0.1	None	for 12 mo	18 mo	0/11 (0)	0/11 (0)	0/11 (0)		
Light duty												
		NS, 9	Whole exhaust	1.1	None		12 mo	0/9 (0)	0/9 (0)	0/9 (0)		
		NS, 11	Whole exhaust	1.1	None		18 mo	0/11 (0)	0/11 (0)	0/11 (0)		
		NS, 5	Whole exhaust	0.5	None	16 hr/day,	6 mo	0/5 (0)	0/5 (0)	0/5 (0)		
		NS, 9	Whole exhaust	0.5	None	6 days/week,	12 mo	0/9 (0)	0/9 (0)	0/9 (0)		
		NS, 5	Whole exhaust	1.8	None		6 mo	0/5 (0)	0/5 (0)	0/11 (0)		
		NS, 6	Whole exhaust	1.8	None		12 mo	0/6 (0)	0/6 (0)	0/6 (0)		
									Primary lung tumors			
Brightwell et	Rat/344	M + F, 260	Clean air	0	None	16 hr/day,	NA		3/260 (1.2)			Tumor
				0	None	5 days/week,						
			exhaust (medium									all rats dying or sacrificed
		M + F, 143	Filtered exhaust (high						0/143 (0)			
												♀ 24/25 (96%)
		M + F, 143	Whole exhaust	0.7	None				1/143 (0.7)			after 24 mo
			Whole exhaust	2.2	None				[c]			12/27 (44%)
		M + F, 143							55/143 (38.5)			after 24 mo
										Squamous cell	tumors	
	Rat/Wistar			0	DPN	19 hr/day,					(84.8)	
(1989a)		F, NS	Whole exhaust		DPN[d]					(4.4)		
		F, NS	Filtered	0	[d]	for 24 to				(46.8)	(67.4)	
						30 mo				(4.4)		
			Clean air	0	DPN						(93.8)	
		F, NS	Whole exhaust		DPN[e]					(16.7)		
		F, NS		0	[e]					[c]	(89.6)	
			exhaust							(14.6)		
Lewis et al.	Rat/F344	M + F, 288	Clean air		None			No tumors			0/192 (0)	
			Whole exhaust		None	5 days/week, 24 mo						

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
								Adenosquamous carcinomas	Squamous cell carcinomas	All tumors		
Takaki et al. (1989) Light-duty engine	Rat/F344	M + F, 123	Clean air	0	None	16 hr/day, 6 days/week, for up to 30 mo	NA	1/23 (0.8)	2/123 (1.6)	1/23 (0.8)	4/123 (3.3)	
		M + F, 123	Whole exhaust	0.1	None			1/23 (0.8)	1/23 (0.8)	1/23 (0.8)	3/123 (2.4)	
		M + F, 125	Whole exhaust	0.4	None			1/25 (0.8)	0/125 (0)	0/125 (0)	1/125 (0.8)	
		M + F, 123	Whole exhaust	1.1	None			0/23 (0)	5/123 (4.1)	0/123 (0)	5/123 (4.1)	
		M + F, 124	Whole exhaust	2.3	None			1/24 (8.1)	2/124 (1.6)	0/124 (0)	3/124 (2.4)	
								Adenomas	Adenocarcinomas	Squamous cell carcinomas	Benign squamous cell tumors	
Heinrich et al. (1995)	Rat/Wistar	F, 220	Clean air	0	None	18 hr/day, 5 days/week, for up to 24 mo	6 mo	0/217 (0)	1/217 (<1)	0/217 (0)	0/217 (0)	
		F, 200	Whole exhaust	0.8	None			0/198 (0)	0/198 (0)	0/198 (0)	0/198 (0)	
		F, 200	Whole exhaust	2.5	None			2/200 (1)	1/200 (<1)	0/200 (0)	7/200 (3.5)	
		F, 100	Whole exhaust	7.0	None			4/100 (4)	4/100 (4)	2/100 (2)	14/100 (14)	Tumor incidences after 30 mo
		F, 100	Carbon black	11.6	None			13/100 (13)	13/100 (13)	4/100 (4)	20/100 (20)	
		F, 100	TiO_2	10.0	None			4/100 (4)	13/100 (13)	3/100 (3)	20/100 (20)	
								Adenomas	Adenocarcinomas	Squamous cell carcinoma	Adeno-squamous carcinoma	Other neoplasms
Nikula et al. (1995)	Rat/F344	M + F, 214[b]	Clean air	0	None	16 hr/day, 5 days/week for up to 24 mo	6 weeks	1/214 (<1)	1/214 (<1)	1/214 (<1)	0/214 (0)	0/214 (0)
		M + F, 210	Whole exhaust	2.5	None			7/210 (3)	4/210 (2)	3/210 (1)	0/210 (0)	0/210 (0)
		M + F, 212	Whole exhaust	6.5	None			23/212 (11)	22/212 (10)	3/212 (1)	1/212 (<1)	0/212 (0)
		M + F, 213	Carbon black	2.5	None			3/213 (1)	7/213 (3)	0/213 (0)	0/213 (0)	1/213 (<1)
		M + F, 211	Carbon black	6.5	None			13/211 (6)	21/211 (10)	3/211 (1)	2/211 (<1)	0/211 (0)
Iwai et al. (1997)	F/344	121, F	Clean air	0	None	NA	NA	5/121(4%) type not stated				
		108, F	Filtered air	0	None	48-56 hr/day	6 mo	2/108(4%) type not stated				Cumulative exposure dose ranged from 154-274 mg/m^3
		153, F	Whole exhaust	3.2-9.4	None	48-56 hr/day	6 mo	53/153(35%) 61.3% adenoma, 25.8% adenocarcinoma, 2.2% benign squamous cell tumor, 7.5% squamous cell carcinoma, 3.2% adenosquamous carcinoma				
Orthoefer et al. (1981) (Pepelko and Peirano, 1983)	Mouse/ Strong A	M, 25	Clean air	0	None	20 hr/day, 7 days/week, for 7 weeks		3/22 (13.6)				0.13 tumors/ mouse
			Whole exhaust	6.4	None		26 weeks	7/19 (36.8)				0.63 tumors/ mouse
			Whole exhaust	6.4	UV irradiated		26 weeks	6/22 (27.3)				0.27 tumors/ mouse
								Lung tumors				

Study	Species/	Sex/total number	atmosphere	Particle concentration (^{3})	treatment	Exposure	Post-exposure	Tumor type and incidence (%)[a]			
	Mouse/ Jackson A	M + F, 40	Clean air		None	7 days/week, for 8 weeks	8 weeks				0.5 tumors/
			Whole exhaust	6.4			8 weeks	11/34 (32.3)			0.4 tumors/ mouse
	Jackson A	F, 60		0	None	7 days/week, for approx.		4/58 (6.9)			mouse
		F, 60	Clean air		Urethan						0.25 tumors/
			Whole exhaust	6.4	None			14/56 (25.0)			0.32 tumors/ mouse
		F, 60		6.4	Urethan			22/59 (37.3)			mouse
		M, 429	Clean air		None						0.23 tumors/
			Whole exhaust	6.4	None			66/368 (17.9)			0.20 tumors/ mouse
	Mouse	M, 458	Clean air	1.5	None			Pulmonary adenomas			
(1982)		M, 18	Clean air		None[k]	7 days/week, for 3 mo		144/458 (31.4)			
								165/485 (34.2)			
								Pulmonary adenoma			
	Mouse/		Clean air	0		20 hr/day,	NA				
(1983)	A/J	M, 388	Whole exhaust		None	7 days/week,		131/388 (33.8)			
White et al.		M, 399		0.75	None			109/399 (27.3)			
			Whole exhaust	1.5		8 mo					
Pepelko and		M + F, 260			None	Continuous	NA	Adenomas	Carcinomas	All tumors	
Peirano (1983)	Sencar		Clean air		BHT				(0.5)		
			Clean air		Urethan[k]			(12.2)		(2.8)	
								(8.1)	(0.9)	(9.0)	
			Whole exhaust		BHT[l]			[c]	(1.0)	[c]	
			Whole exhaust		[l]			(5.4)		(8.1)	
									(2.6)		

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						10/80 (12.5)	0.14
			Whole exhaust		(darkness)						22/250 (0.10)	0.10
			Clean air		Urethan[m]						66/75 (88)	2.80
			Whole exhaust		Urethan[m]						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,	NA	9/84 (11)	2/84 (2)	—	11/84 (13)	
		M + F, 93	Filtered exhaust		None	5 days/week for up to		11/93 (12)	18/93 (19)[c]	—	29/93 (31)[c]	
		M + F, 76	Whole exhaust		None	30 mo		11/76 (15)	13/76 (17)[c]	—	24/76 (32)[c]	
									Adenoma	Adenocarcinoma		
Takemoto et al. (1986)	Mouse/IRC	M + F, 45	Clean air	0	None	4 hr/day,	NA					
		M + F, 69	Whole exhaust	2-4	None	4 days/week, for 19-28 mo						
	Mouse/ C57BL	M + F, 12	Clean air	0	None	4 hr/day,	NA		3/45 (6.7)	1/45 (2.2)		
		M + F, 38	Whole exhaust	2-4	None	4 days/week for 19-28 mo			6/69 (8.7)	3/69 (4.3)		
Heinrich et al. (1995)	Mouse/ C57BL/6N	F, 120	Clean air	4.5	None	18 hr/day,	6 mo		1/12 (8.3)	0/12 (0)		5.1% tumor rate
						5 days/week,			8/38 (21.1)	3/38 (7.9)		
		F, 120	Whole exhaust		None	for up to 21 mo						8.5% tumor rate
		F, 120	Particle-free exhaust		None							3.5% tumor rate
									Adenomas	Adenocarcinomas		
	Mouse/ NMRI	F, 120	Clean air	0	None	18 hr/day,	9.5 mo					
		F, 120	Whole exhaust	4.5	None	5 days/week			(25)	(15.4)		
			Carbon black	11.6	None	for up to			(21.8)	(15.4)		
			TiO_2	10	None	13.5 mo			(11.3)	(10)		
									(11.3)	(2.5)		
	Mouse/ NMRI	F, 120	Clean air	4.5	None	18 hr/day,	None		(25)	(8.8)		
		F, 120	Whole exhaust		None	5 days/week,			(18.3)	(5.0)		
		F, 120	Particle-free exhaust		None	23 mo			(31.7)	(15)		

Table 7-3. Summary of animal inhalation carcinogenicity studies (continued)

	Species/ strain	number	Exposure	Particle concentration (mg/m)	Other	Exposure protocol	exposure observation	[a]				Comments
								adenomas	Multiple	Adenomas/ carcinoma	bronchiolar adenoma	bronchiolar carcinoma
	Mouse/CD-1	[b]	Clean air		None	7 hr/day, 5		1/157 (0.6)	2/157 (1.3)		10/157 (6.4)	7/157 (4.5)
(1996)		M + F, 171		0.35	None			2/171 (1.2)		1/171 (0.6)	16/171 (9.4)	
			Whole exhaust	3.5		for up to 24			1/155 (0.6)	0/155 (0)		6/155 (3.9)
		M + F, 186	Whole exhaust		None	mo		0/186 (0)	0/186 (0)		10/186 (5.4)	4/186 (2.2)
										Squamous		
									Adenocarcinoma	tumors	All tumors	
	Hamster/	M + F, 96	Clean air		None	19 hr/day		0/96(0)	0/96(0)	0/96	0/96(0)	
(1986a,b)		M + F, 96	Filtered exhaust					0/96(0)	0/96(0)	0/96	0/96(0)	
						for up to						
				4	None	30 mo						
									Primary lung tumors			
				0	None	16 hr/day,	NA		7/202 (3.5)			Respiratory
al.	Syrian	M + F, 202	Clean air	0	DEN	5 days/week,						tract tumors not
(1989)	Golden	M + F, 104	Filtered		DEN[j]				9/104 (8.7)			
												exhaust
			(medium dose)									exposure for
				0	[j]				2/101 (2.0)			groups
			exhaust (high dose)									
		M + F, 101		0.7	DEN							
		M + F, 102	Whole exhaust		DEN[j]				4/101 (3.9)			
			Whole exhaust	6.6	[j]				1/204 (0.5)			
		M + F, 204		0	None							
			exhaust (high dose)									
		M + F, 203										

[a]

[b]Number of animals examined for tumors.

Significantly different from clean air controls.

[d]

[e]Dipentylnitrasamine; 12.5 mg/kg/week s.c. during first 25 weeks of exposure.

Splenic lymphomas also detected in controls (8.3%), filtered exhaust group (37.5%) and whole exhaust group (25%).

5.3% incidence of large cell carcinomas.

[h]

[i]Includes adenomas, squamous cell carcinomas, adenocarcinomas, adenosquamous cell carcinoma, and

[j]4.5 mg/diethylnitrosamine (DEN)/kg, s.c., 3 days prior to start of inhalation exposure.

Single i.p. dose 1 mg/kg at start of exposure.

[l]

to 52.

[m]

[3]from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents [3].

120-121 males and 71-72 females examined histologically.

[o]

exposure.

NS = Not specified.

Table 7-4. Tumor incidences in rats following intratracheal instillation of diesel exhaust particles (DPM), extracted DPM, carbon black (CB), benzo[*a*]pyrene (B*a*P), or particles plus B*a*P

Experimental group	Number of animals	Total dose	Animals with tumors (percent)	Statistical significance[a]
Control	47	4.5 mL	0 (0)	-
DPM (original)	48	15 mg	8 (17)	< 0.01
DPM (extracted)	48	30 mg	10 (21)	< 0.001
DPM (extracted)	48	15 mg	2 (4)	NS
CB (printex)	48	15 mg	10 (21)	< 0.001
CB (lampblack)	48	14 mg	4 (8)	NS
B*a*P	47	30 mg	43 (90)	< 0.001
B*a*P	48	15 mg	12 (25)	< 0.001
DEP + B*a*P	48	15 mg + 170 µg B*a*P	4 (8)	NS
CB (printex) + B*a*P	48	15 mg + 443 µg B*a*P	13 (27)	< 0.001

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.

Table 7-6. Tumor incidence and survival time of rats treated by surgical lung implantation with fractions from diesel exhaust condensate (35 rats/group)

Material portion by weight (%)	Dose (mg)	Median survival time in weeks (range)	Number of carcinomas[a]	Number of adenomas[b]	Carcinoma incidence (%)
Hydrophilic fraction (I) (25)	6.7	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
Benzo[*a*]pyrene	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.

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.

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

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Mauderly et al. (1987)	35, 30	4.20042004e+15	0	0	147014700298 20	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	740021800617 00	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) (Light-duty engine)	96, 30	1.15201152e+49	0	0	1.1524 60813e+37	3.3
	96, 30		0.1	9.6		2.4
	96, 30		0.4	38.4		0.8
	96, 30		1.1	105.6		4.1
	96, 30		2.3	220.8		2.4
(Heavy-duty engine)	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 diesel exhaust (continued)

Study	Exposure rate/duration (hr/week, mo)	Total exposure time (hr)	Particle concentration (mg/m^3)	Cumulative exposure ($mg \cdot hr/m^3$)		Tumor incidence (%)[a]
				Per week	Total	
Brightwell et al. (1989)	80, 24	7.6807681e+15	0	0	537616896506	1.2
	80, 24		0.7	56.0	88	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	210063001260	0
	140, 15		0.25	35.0	0	3.3
	140, 15		0.75	105.0		10.0
	140, 15		1.5	210.0		3.3
Iwai et al. (1986)	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			

Table 7-9. Evaluations of diesel exhaust 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)
U.S. DHHS (2000)	"Elevated lung cancer in occupationally exposed groups"	"Supporting animal and mechanistic data"	Reasonably anticipated to be a carcinogen

[a] Not applicable.

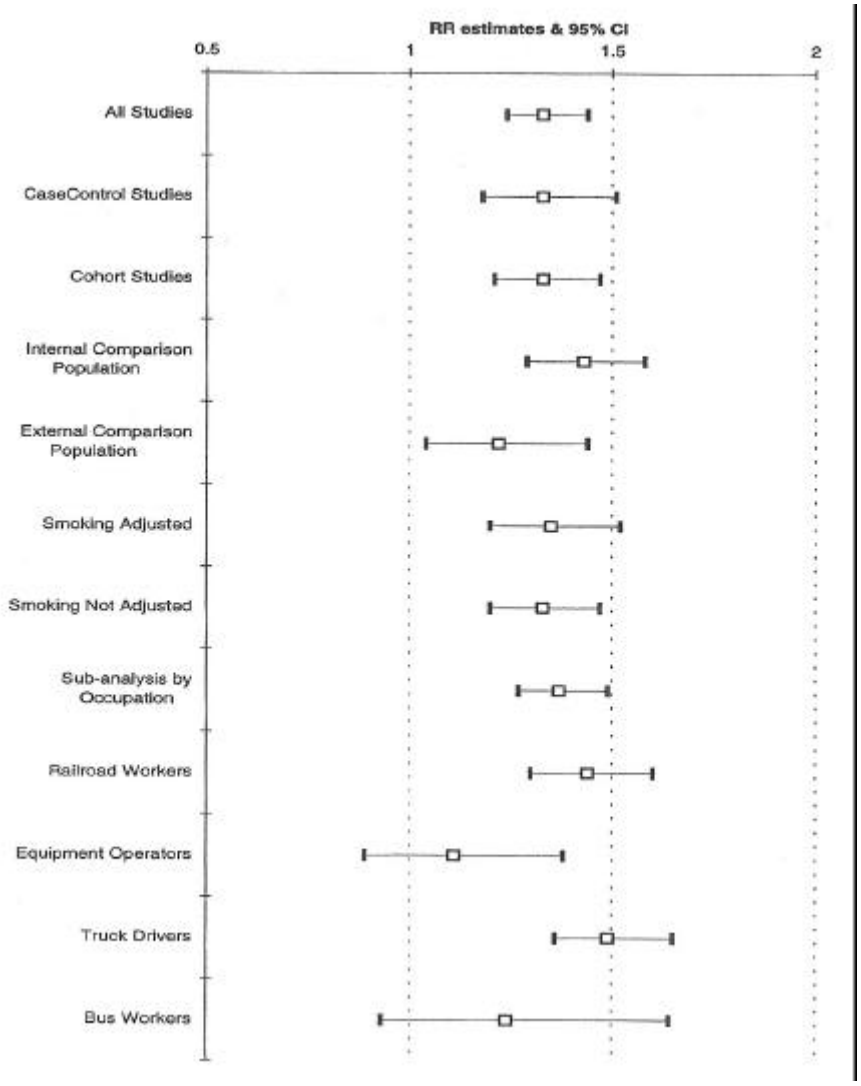
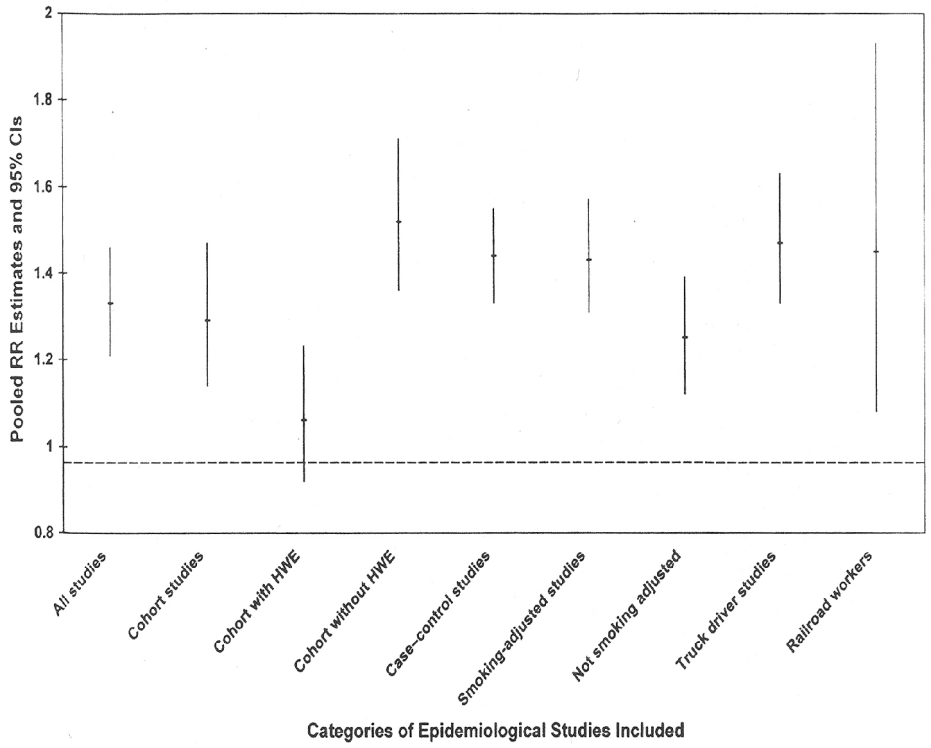


Figure 7-1. Pooled relative risk estimates and heterogeneity-adjusted 95% confidence intervals for all studies and subgroups of studies included in the meta-analysis.

Source: Bhatia et al., 1998.



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

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

Source: Lipsett and Campleman, 1999.

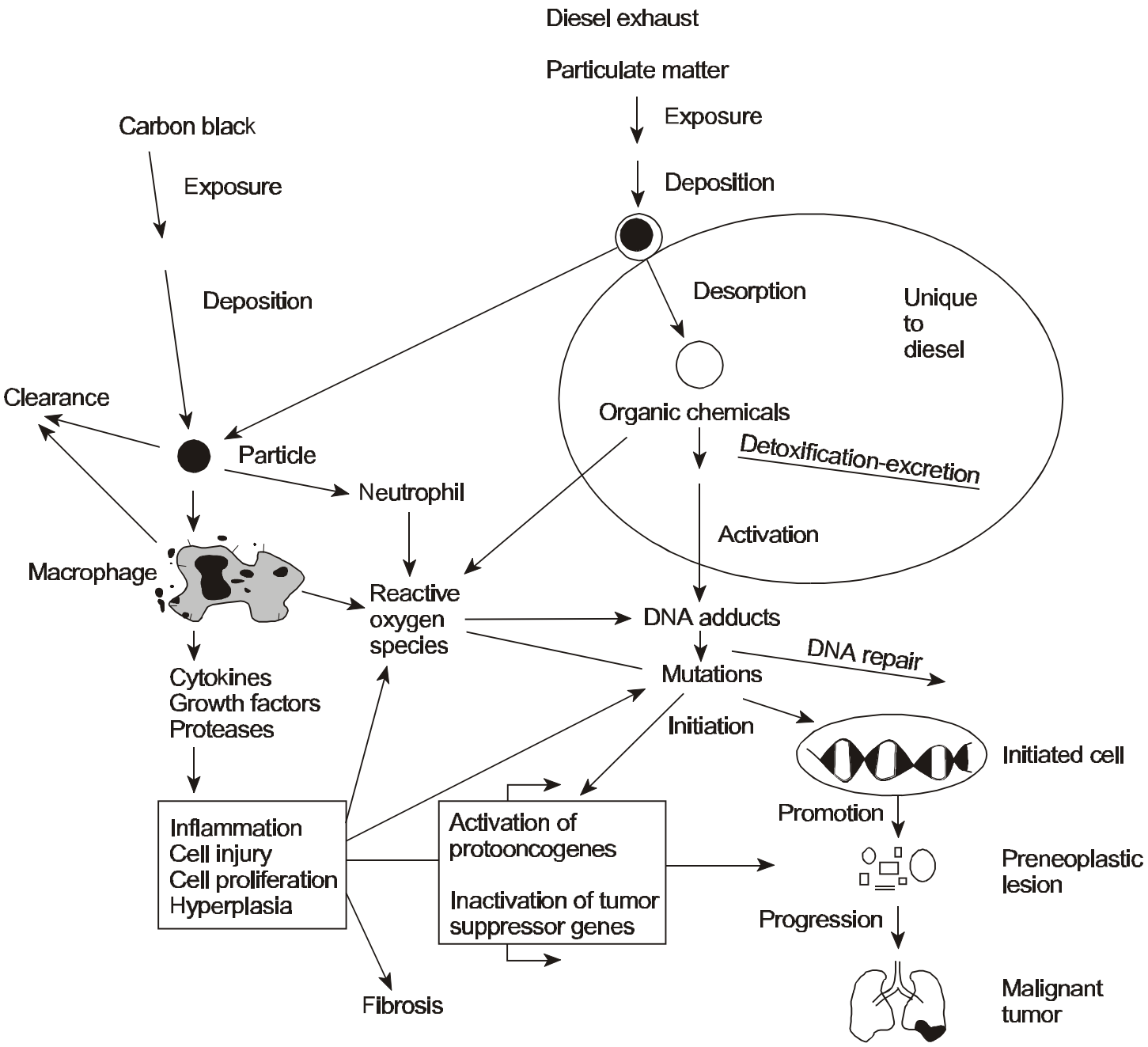


Figure 7-3. Pathegenesis of lung disease in rats with chronic, high-level exposures to particles.

Source: Modified from McClellan, 1997.

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