Part B: Health Risk Assessment for Diesel Exhaust

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Part B:

HEALTH RISK ASSESSMENT FOR DIESEL EXHAUST

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Prepared by:

Stanley V. Dawson, D.Sc.
George V. Alexeeff, Ph.D.
John D. Budroe, Ph.D.
Sharan Campelman, Ph.D., M.P.H.
James F. Collins, Ph.D.
Jefferson R. Fowles, Ph.D.
David A. Holtzman, M.P.H.
Richard F. Lam, Ph.D.
David C. Lewis, Ph.D.
Michael J. Lipsett, M.D.
Melanie A. Marty, Ph.D.
Frank J. Mycroft, Ph.D.
Bart D. Ostro, Ph.D.
Judith S. Polakoff, M.S.
Kathleen Vork, M.P.H.

Office of Environmental Health Hazard Assessment

Allan H. Smith, M.D., Ph.D.
Michael N. Bates, Ph.D.
Helen M. Goeden, Ph.D.
School of Public Health

University of California, Berkeley

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PREFACE

This draft of Part B, "Health Risk Assessment for Diesel Exhaust," was prepared by OEHHA for review by the public and the Scientific Review Panel of the Air Resources Board. This draft builds upon previous drafts (June 1994, March 1997, February 1998), which were the subjects of extensive review and comment. The new draft has been revised in response to the comments received from the public and the Scientific Review Panel. This draft has also been updated through March 1998 to remain current with the information available. This document has undergone extensive internal and external review by scientists. The summary of the OEHHA assessment and its conclusions is found in Chapter 1. Chapter 2 provides a description of the contents of this draft and indicates where the substantial new analyses have been incorporated.

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1.0 SUMMARY

Diesel exhaust consists of a complex mixture of substances formed in the combustion processes of a diesel engine. The mixture includes compounds in a vapor phase and very fine particles with a carbon core coated by condensed organic compounds. This report characterizes the potential for diesel exhaust to affect human health, and the associated scientific uncertainties. It considers both carcinogenic and noncarcinogenic effects. The main conclusions concern the potential of diesel exhaust to cause lung cancer in humans and the likely magnitude of the cancer risk.

1.1 NON-CANCER HEALTH EFFECTS

This document, aside from Appendix B, focuses on the health effects specifically associated with diesel exhaust. An extensive series of studies has provided epidemiological evidence of the adverse health effects of airborne particles in general on human health. Such particles include the particulate matter in diesel exhaust. The general particle effects observed include both increased morbidity and mortality, and the non-cancer effects are both acute and chronic. Appendix B includes a brief overview of the literature on the health effects of ambient particulate matter. As with any other kind of particle, the comprehensive information available on the serious health effects attributable to particulates in general need to be considered by risk managers addressing diesel exhaust. The final United States Environmental Protection Agency (U.S.EPA) staff paper for PM-10 provides estimates of the mortality risk from particulate matter in Southern California (USEPA, 1996).

1.1.1 RESPIRATORY HEALTH EFFECTS

Epidemiological studies reviewed in this chapter provide evidence which suggests that diesel exposed workers had increased frequency of bronchitic symptoms, cough and phlegm, wheezing, and decrement in lung function. However, confounding factors present during exposure often obscured the exposure-effect relationships in these studies.

The inhalation or direct application of diesel into the respiratory tract of animals in acute and subchronic studies induced inflammatory airway changes, lung function changes, and increased susceptibility of exposed animals to lung infection. The morphological effects observed in the lungs of animals in chronic inhalation exposures are mainly related to chronic inflammatory responses. These changes include thickening of the alveolar epithelium, increase in lung weight, infiltration of macrophages, fibroblasts and proteins into the alveolar septa, and glandular metaplasia.

1.1.2 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

Studies on induced heritable point mutations and sperm abnormalities following diesel exhaust exposure were negative in rats, mice, and monkeys, though sperm anomalies were noted in exposed hamsters. An observed decrease in the number of corpora lutea following diesel exhaust exposure in one study suggests potential impact on female reproduction in mice. Teratogenic

effects were not observed in rabbits. Some evidence of neurobehavioral effects in rat pups is reported. Data of the effects of diesel exhaust exposure on female reproductive capacity are limited but potential effects on corpora lutea and mating period have been suggested in laboratory rodents.

No teratogenic effects of diesel exhaust exposure were shown in rabbits. Delayed ossification of the thoracic region has been noted in rats following exposure to very high exposure levels. Exposure to diesel exhaust during the neonatal developmental period of rodents induces neurobehavioral and neurophysiological effects, but does not affect general lung development. Other organ systems have not been evaluated.

Generational studies conducted in rodents revealed that inhalation exposure to diesel exhaust causes increases in lung weight in all generations examined. Evaluation of other parameters produced inconclusive results.

The available literature does not provide sufficient information to determine whether or not diesel exhaust exposure induces reproductive, developmental or teratogenic effects in humans.

1.1.3 IMMUNOLOGICAL EFFECTS

There are a number of review articles which postulate that air pollution, particularly diesel exhaust particles, plays a role in the increasing prevalence of asthma and other allergic respiratory disease (Albright and Goldstein, 1996; Peterson and Saxon, 1996; Devalia *et al*., 1997). The discovery of the role of diesel particulates and their PAH fraction in augmentation of allergic responses to specific antigens in humans and animals is relatively new, and many of the studies discussed in Chapter 4 are comparatively recent (Peterson and Saxon, 1996; Diaz-Sanchez, 1997).

Diesel exhaust exposure can result in measurable increases in IgE and IgG antibody production, perturbed immunological cytokine regulation, localized inflammation and eosinophilic infiltration in lung and respiratory tract tissues, particularly when the exposure accompanies other known respiratory allergens. In human subjects and in human cells, diesel exhaust particulate (DEP) stimulated IgE antibody production and increased mRNA for the pro-inflammatory cytokines. Co-exposure to DEP and ragweed pollen was reported to significantly enhance the IgE antibody response relative to ragweed pollen alone. DEP also enhanced the IgE antibody and cytokine production response to ovalbumin and Japanese cedar pollen in animal models and increased nasal hyperresponsiveness to histamines. There is some evidence that production of reactive oxygen species may be involved in the asthma-like symptoms produced in mice by DEP exposure.

Although none of the evaluated studies have been designed to yield quantitative estimates of diesel particle concentrations for the purposes of determining a reference exposure level, the potential relevance of these immunological endpoints to public health is very high, due to reports of large numbers of individuals with respiratory allergies and asthma in urban areas (Burney *et*

al., 1990; Corbo *et al*., 1993; Emanuel, 1988; Strachan and Anderson, 1992; Frew and Salvi, 1997).

1.1.4 QUANTITATIVE NON-CANCER ASSESSMENT

The available data from studies of humans exposed to diesel exhaust are not sufficient for deriving a non-cancer health risk guidance value. While the lung is the major target organ for diesel exhaust, studies of the gross respiratory effects of diesel exhaust in exposed workers have not provided sufficient exposure information to establish a non-cancer health risk guidance value for respiratory effects. Most of the epidemiologic studies did not find an excess of chronic respiratory disease associated with diesel exhaust. However, these studies all had limitations, such as small numbers of subjects, limited exposure information, and insensitive measures, which clearly reduced their ability to detect adverse effects. Although recent preliminary studies of human allergic responses indicate that diesel exhaust particles have specific immunological properties that may exacerbate existing respiratory allergies, OEHHA agrees with the U.S.EPA on the use of animal toxicology data to develop non-cancer health risk guidance values.

In June, 1993, the U.S.EPA determined an inhalation Reference Concentration (RfC) of 5 $\mu g/m^3$ for non-cancer effects of diesel exhaust (Appendix A). (The U.S.EPA RfC is currently under review by the U.S.EPA.) The RfC of a chemical is an estimate, with an uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups, that is likely to be without appreciable risk of deleterious effects during a lifetime of exposure. The RfC was derived by applying uncertainty factors to a no observed adverse effect level (NOAEL).

The NOAEL and the lowest observed adverse effect level (LOAEL) were obtained from the chronic study of Ishinishi *et al* (1988). These levels are based on histopathological changes observed in the lungs of Fischer rats. Adverse effects were observed in rats exposed to 960 $\mu g/m^3$; no adverse effects were observed at 460 $\mu g/m^3$. The human equivalent concentrations (HEC) of 155 $\mu g/m^3$ for the NOAEL and 300 $\mu g/m^3$ for the LOAEL resulted from a theoretical model of lung burden per unit alveolar surface area and included an adjustment for hours of exposure. The histopathological changes include an accumulation of particle-laden macrophages associated with bronchiolization of alveolar ducts in which bronchiolar epithelium replaced alveolar epithelium. The investigators also observed proliferation of bronchiolar epithelium and of Type II alveolar cells.

The U.S.EPA derived the RfC by dividing the NOAEL(HEC) by an uncertainty factor of 30. The uncertainty factor of 30 reflects a factor of 10 to protect sensitive individuals and a factor of 3 to allow for interspecies extrapolation. The U.S.EPA did not use the customary value of 10 for interspecies extrapolation because dosimetric adjustments based on a particle deposition and retention model were applied.

The studies used to support the RfC represent three research programs that performed well-conducted chronic studies with adequate numbers of animals. The identified LOAELs and NOAELs are consistent across studies. The RfC data base contains 10 chronic studies as well as

developmental and reproductive studies. High confidence in the RfC follows from these considerations.

The World Health Organization (WHO) and OEHHA have conducted further analyses of the dose-response relationships for several of the non-cancer, adverse effects of chronic exposures to diesel exhaust on rat lung. These analyses gave a range of health risk guidance values of 2 to 21 $\mu g/m^3$ and support the adoption of 5 $\mu g/m^3$ which is also the 1993 U.S.EPA RfC.

An inhalation Reference Exposure Level for acute effects has not been calculated.

In conclusion, there is evidence to suggest that human exposure to diesel exhaust causes chronic respiratory symptoms and contributes to the recent increase in allergic respiratory diseases. Results from animal studies provide support for this conclusion. The human health effects data currently available do not themselves allow for a quantitative derivation of a chronic inhalation REL. The OEHHA concurs with the U.S. EPA that the chronic rat study by Ishinishi *et al*. (1988) is the most appropriate study for this purpose and recommends 5 $\mu g/m^3$ as the chronic REL for diesel exhaust.

1.2 GENOTOXICITY

Studies of diesel exhaust-induced genotoxicity aid in the assessment of the cancer risk posed by exposure to diesel exhaust by suggesting mechanisms of carcinogenicity and other genetically influenced processes caused by DNA-reactive agents, and by suggesting the general form for the quantitative cancer models. Altered oncogene or lost tumor-suppressor-gene function may be in part responsible for the carcinogenicity of DNA-reactive agents (Tong *et al*., 1989; Marshall *et al*., 1991; Solomon *et al*., 1991; Weinberg *et al*., 1991).

Much of the information regarding genotoxicity has been obtained using diesel exhaust particles or extracts of diesel exhaust particles. Diesel exhaust particles or their extracts are mutagenic in bacteria (*Salmonella typhimurium* and *E. coli*) and in several mammalian cell systems (CHO, V79, BALB/c3T3, L5718Y mouse lymphoma, human lymphoblasts). Diesel exhaust particles or their extracts induce chromosome aberrations, aneuploidy, and sister chromatid exchange in rodent and human cells in culture. Diesel exhaust particles and their extracts are also capable of inducing cell transformation. Diesel exhaust particles or their extracts can also produce superoxide and peroxide radicals and inhibit the antioxidant enzymes responsible for radical scavenging. Diesel exhaust particles have also been shown to cause an increase in 8-hydroxydeoxyguanosine (8-OHdG) adducts in calf thymus DNA *in vitro* and in lung DNA from mice exposed *in vivo* by intratracheal instillation. Both diesel exhaust particle extracts and the semivolatile phase of diesel exhaust have dioxin receptor (Ah receptor) binding affinity. Exposure to diesel exhaust particulate matter can cause unscheduled DNA synthesis in vitro in mammalian cells. DNA adducts have been isolated from calf thymus DNA *in vitro* and mouse lung DNA following intratracheal instillation.

Some information regarding genotoxicity also has been obtained directly from diesel exhaust exposures. Whole diesel exhaust has been demonstrated to induce gene mutations in two strains of *Salmonella*. Inhalation exposure to diesel exhaust results in DNA adduct formation in rodents and monkeys. Increased levels of human peripheral blood cell DNA adducts are associated with occupational exposure to diesel exhaust. The genotoxic effects of diesel exhaust may be involved in the initiation of pulmonary carcinogenesis in humans.

Diesel exhaust clearly contains genotoxic substances. Furthermore, diesel exhaust particles and diesel exhaust extracts have been established to be genotoxic. The bioavailablity of these genotoxins has been questioned. Several lines of evidence suggest bioavailability. First, the *in vitro* genotoxic activity of diesel exhaust particulates dispersed in pulmonary surfactant exhibited similar activity to particulates extracted with dichloromethane. Second, inhalation exposure of rats and monkeys to diesel exhaust results in DNA adduct formation and in vitro exposure of rat tissues to diesel exhaust induces unscheduled DNA synthesis. Third, DNA adducts have been associated with occupational exposure to diesel exhaust. Fourth, urinary metabolites of PAHs have been found following exposure of rats to diesel exhaust. Preliminary evidence indicates the same may be true for humans. Consequently, it appears that organic chemicals adsorbed onto the particles, particularly the genotoxic components, are likely to be bioavailable in humans.

1.3 CARCINOGENICITY

1.3.1 ANIMAL TESTS

Three animal species -- rats, mice and Syrian hamsters -- have been adequately studied for the carcinogenic effects of inhalation of diesel exhaust. A study by Lewis *et al*. (1986; 1989) in cynomolgus monkeys was negative. The duration of exposure in Lewis *et al*. (1986) was considerably less than lifetime, rendering it unsuitable for a determination of carcinogenicity.

Results of inhalation bioassays in the rat have demonstrated the carcinogenicity of diesel exhaust in test animals. Until the early-1980's, inhalation studies in rodents examining the potential carcinogenic effect of diesel exhaust emissions failed to demonstrate any statistically significant increase in the incidence of pulmonary tumors in exposed animals. More recent studies utilizing higher exposure levels and/or longer observation periods (>24 months) have consistently demonstrated significant increases in pulmonary tumors in rats. All seven studies in rats using exposure concentrations of greater than or equal to 2.2 mg/m^3 of whole diesel exhaust (time-weighted average equivalent to approximately 1 mg/m^3), and using observation periods of approximately 2 years or longer, reported statistically significant excesses of lung tumors (Iwai *et al*. 1986, Heinrich *et al*. 1986b, Mauderly *et al*. 1987a, Brightwell *et al*. 1986; Ishinishi *et al*. 1986a; Heinrich *et al*., 1995; Nikula *et al*., 1995). Nonsignificant increases in lung tumor incidence in rats have been observed at diesel exhaust particulate concentrations between 0.35 and 2.2 mg/m^3. These studies also provided data that can be used for a quantitative risk assessment (QRA). Four studies in rats at lesser exposures or with shorter observation periods were inconclusive or negative. The finding that the rat lung also develops cancers in response to other particulates, carbon black and titanium dioxide, indicates that diesel exhaust particulate matter may importantly contribute to the carcinogenicity of diesel exhaust in the rat. Studies in

mice have mixed results. Unfiltered diesel exhaust significantly increased lung tumor incidence in female Strong A mice, female Sencar mice, and female NMRI mice (Pepelko and Peirano, 1983; Heinrich *et al*, 1986a). In female Strong A mice, however, the highest exposure resulted in a decrease in lung tumor incidence relative to controls (Pepelko and Peirano, 1983). Exposure of female NMRI mice to filtered diesel exhaust has produced both positive and negative results (Heinrich *et al.,* 1986b; Heinrich *et al.,* 1995). Other studies in mice are negative. All three studies in hamsters were negative. Negative results in hamsters are consistent with the finding that, unlike rats, hamsters do not demonstrate increases in DNA adduct formation following a 12-week exposure to diesel exhaust particulate matter (see Section 5.4).

The mechanisms by which diesel exhaust induces lung tumors in rats are not certain. Several hypotheses have been proposed. One hypothesis invokes the genotoxicity of the compounds condensed on the surfaces of the diesel exhaust particle. This hypothesis suggests the operation of a general mechanism shared with humans and the absence of a dose-response threshold. Another hypothesis is that diesel exhaust leads to oxidative damage to DNA by a mechanism other than particle-induced inflammation. A third hypothesis is that the particulate nature of diesel exhaust is responsible for its carcinogenicity. The inflammatory response to any fine particle could, at high lung burdens, cause cellular proliferation and thereby increase the chance of DNA replication before repair. This last mechanism could possibly operate in humans but may suggest a dose-response threshold. More than one mechanism may be involved as there is some evidence for each hypothesis.

1.3.2 COMPONENTS OF DIESEL EXHAUST

Many carcinogenic compounds are found in diesel exhaust. Compounds found in the vapor phase include benzene, formaldehyde, 1-3-butadiene, and ethylene dibromide. At least 16 hydrocarbons that are classified as possibly carcinogenic (IARC Classification 2B) to humans are adsorbed on the exhaust particles. Additionally, benzo[a]pyrene, benz[a]anthracene, and dibenz[a,h]anthracene, which are classified as probably carcinogenic to humans (IARC Classification 2A), are adsorbed on the particles (IARC, 1989). A report of work at the U.S.EPA surmised that the total carcinogenic effect estimated for all these compounds does not account for all of the carcinogenic effect of the whole diesel exhaust (Pepelko and Ris, 1992). In this regard, Heinrich *et al*. (1995) and Nikula *et al*. (1995) report the carcinogenicity of carbon black in the form of particles which are similar to the carbon core of the diesel exhaust particles but which carry negligible adsorbed material.

While the vapor includes numerous cancer causing compounds, several animal studies found tumors only from exposure to diesel exhaust particles. Three rat bioassays (Iwai *et al*., 1986; Heinrich *et al*., 1986b; Brightwell *et al*., 1989) found that filtration of the exhaust, in order to remove particles, eliminated any significant tumor response to exposure. In contrast, a study in one strain of mice found similar increased incidences of malignant tumors in filtered and unfiltered exhaust groups (Heinrich *et al*., 1986b). However, a similar study in the mouse (Heinrich *et al*, 1995) did not find such an effect.

A general issue with regard to characterizing the toxicity of diesel exhaust is the variability of exhaust composition among types of engines and over different driving (or other use) conditions. However, findings suggest that variability in toxicity may be small when the health evaluation is based on concentration of particulate matter.

The report here measures the carcinogenic effect of whole diesel exhaust against the mass per volume of air of the diesel particulate matter. Therefore, the particle mass serves as a surrogate measure for the whole (particulate and gaseous phases) diesel exhaust exposure.

1.3.3 EPIDEMIOLOGICAL STUDIES

Over 30 epidemiological studies have investigated the potential carcinogenicity of diesel exhaust. The epidemiological evidence primarily concerns cancer of the lung. The question whether diesel exhaust causes lung cancer has been addressed by both industry-based cohort studies and case-control studies as well as population-based studies of lung cancer. Because there are no epidemiological studies involving industrial hygiene measurements concurrent with the exposure of the study populations, exposure has typically been defined by the surrogate measures of usual occupation or job classification within an industry.

There is a considerable degree of consistency in finding elevated, although not always statistically significant, lung cancer risks in workers likely to have been exposed to diesel exhaust within several industries. General population-based case-control studies identified statistically significant increases in lung cancer risk for truck drivers (Hayes *et al*. 1989; Swanson *et al*. 1993), railroad workers (Swanson *et al*., 1993), heavy equipment operators (Boffetta *et al*., 1988), and for self-reported diesel exhaust exposure in general (Siemiatycki *et al*., 1988). All of these significantly elevated estimates were adjusted for smoking. Industry-specific studies, both of case-control and cohort design, identified statistically elevated lung cancer risk for truck drivers (Ahlberg *et al*., 1981; Rafnsson and Gunnarsdottir, 1991; Guberan *et al*., 1992; Hansen *et al*., 1993), professional drivers (Benhamou *et al*., 1988; Pfluger and Minder, 1994) and railroad workers (Howe *et al*., 1983; Garshick *et al*., 1987a; Garshick *et al*., 1988), with a minority of these studies adjusting for smoking (Benhamou *et al*., 1988; Pfluger and Minder, 1994; Garshick *et al*., 1987a).

In order to summarize quantitatively the overall and occupation-specific risks from the epidemiological studies, a meta-analysis was conducted (see Appendix C). This meta-analysis provides strong support for the hypothesis that occupational exposure to diesel exhaust is associated with an increased risk of lung cancer. Pooled relative risk estimates from 30 studies clearly reflect the existence of a positive relationship between diesel exhaust and lung cancer in a variety of diesel-exposed occupations, which is supported when the most important potential confounder, cigarette smoking, is measured and controlled. Another independently conducted meta-analysis was published in January 1998, also reporting a persistent positive relationship between occupational diesel exhaust exposure and lung cancer that could not be attributed to potential confounding by cigarette smoking (Bhatia *et al*., 1998).

The relative risk estimates obtained in these analyses are still generally low, since the estimates are less than two. Relative risk estimates of this order potentially weaken the evidence of causality, due to the increased possibility of unknown sources of bias or confounding producing the findings. However, associations of this magnitude can serve as the basis for causal inference (e.g., active smoking and heart disease or environmental tobacco smoke exposure and lung cancer) provided that other criteria are met.

A large majority of studies reviewed here indicated a positive association between lung cancer and occupational exposure to diesel exhaust. Several studies which accounted for at least one of the two principal confounders, smoking and exposure to asbestos, found significantly elevated risks, especially after longer-term exposures (Garshick *et al*., 1987; Garshick *et al*. 1988; Gustavson *et al*., 1990; Hayes *et al*., 1989; Swanson *et al*., 1993). Additionally, the quantitative summary provided by the meta-analysis demonstrated not only that the increases in lung cancer risk remained after stratification by smoking or occupation, but in several instances increased. For example, the pooled relative risk estimate for railroad workers increased from 1.45 to 1.68 (95% CI = 1.28-2.19), when only smoking-adjusted risk estimates were considered.

1.3.4 CONCLUSIONS CONCERNING CAUSAL INFERENCE

Epidemiological studies provide evidence consistent with a causal relationship between occupational diesel exhaust exposure and lung cancer. The evidence linking diesel exposure and bladder cancer is not as extensive or compelling. The majority of studies examining the diesel exhaust-lung cancer association have reported elevated estimates of relative risk, many of which are statistically significant. The consistency of these findings is unlikely to be due to chance. Moreover, with the possible exception of some studies that did not take smoking into account, the results are unlikely to be explained by confounding or bias. This is reinforced by the results of a meta-analysis undertaken by OEHHA staff (Appendix C, summarized in Section 6.2.2), in which statistically significant pooled estimates of relative risk persisted through numerous subset and sensitivity analyses. For example, the pooled relative risk estimate for studies that adjusted for smoking was 1.43 (95% CI = 1.31-1.57). In addition, several studies show clear exposure-response relationships. The strength of the associations reported is typically within the range considered “weak” in epidemiology (i.e., estimates of relative risk between 1 and 2); nonetheless, this is not a bar to causal inference as long as other criteria are met, as discussed in Section 6.2.4.

Finally, it is biologically plausible because of its mutagenic and carcinogenic constituents that exposure to diesel exhaust would increase the risk of lung cancer. Therefore, a reasonable and likely explanation for the increased risks of lung cancer observed in the epidemiologic studies is a causal association between diesel exhaust exposure and lung cancer.

This conclusion can be compared to those of the several prominent bodies that have assessed the carcinogenic potential of diesel exhaust. The National Institute of Occupational Health and Safety (NIOSH) first recommended that whole diesel exhaust be regarded as a potential occupational carcinogen based upon animal and human evidence in 1988. The International Agency for Research on Cancer (IARC, 1989) concluded that there is sufficient evidence for the carcinogenicity of whole diesel engine exhaust in experimental animals and that there is limited

evidence for the carcinogenicity of whole diesel exhaust in humans. On that basis, IARC concluded that diesel engine exhaust is probably carcinogenic to humans and classified diesel exhaust in Group 2A. Based upon the IARC findings, in 1990, the State of California under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) identified diesel exhaust as a chemical known to the State to cause cancer. The U.S.EPA has twice proposed a conclusion similar to IARC in their draft documents. In 1990, they proposed to classify diesel emissions into their equivalent category B-1 (U.S.EPA, 1990; Pepelko and Ris, 1992). The 1994 draft U.S.EPA document concluded similarly that there was sufficient animal evidence of carcinogenicity and that the human evidence was limited (U.S.EPA, 1994). The 1998 draft U.S.EPA document concluded that based on the human evidence alone, diesel exhaust is close to being a human carcinogen (U.S. EPA 1998).

The Health Effects Institute (HEI) and the World Health Organization (WHO) also evaluated the carcinogenicity of diesel exhaust (HEI, 1995; WHO, 1996). The HEI and WHO both found that the epidemiological data are consistent in showing weak associations between exposure to diesel exhaust and lung cancer. The HEI and others have also considered that the absence of reliable exposure data, inability to control for some confounders, and questions about one's ability to estimate a dose-response relationship in the epidemiological studies limits the ability to use them for quantitative risk assessment. The HEI found that the carcinogenicity of diesel exhaust had been convincingly demonstrated in rats. However, the HEI also concluded that the rat lung response was likely related to an overload of the rat clearance mechanism. HEI found that there was evidence indicating the effect was specific to the rat and therefore cautioned against extrapolating the rat findings to humans exposed at ambient levels.

1.4 QUANTITATIVE RISK ASSESSMENT

The quantitative risk assessment (QRA) uses the carcinogenicity data from two human studies to predict risks of cancer in humans that are subject to ambient exposures of diesel exhaust. In addition, QRA of the rat data was also conducted for comparison but the results are not used in the final range of risks. This assessment predicts risk in relation to the mass of diesel particulate matter, which serves as a practical surrogate for the source of carcinogenicity in diesel exhaust. This choice also allows direct use of common measurements of air pollution in the particle phase.

This assessment follows California guidelines in reporting unit risks as 95% upper confidence limits (UCLs) of the mathematical model. In the rat-based predictions, these unit risks are obtained from results of modeling the rat data first and then scaling the results to humans.

In addition to predicting risk based simply on cumulative exposure to diesel exhaust particles from human and rat data, this assessment also predicts risk based on cumulative exposure to the burden of particles in the rat lung. Toxicokinetic data and models are required for this prediction.

1.4.1 TOXICOKINETICS

The biological fate of the inhaled particle-associated compounds is influenced by distribution, clearance, and metabolism in the body, particularly in the lung. In turn each of these parameters can be influenced by the characteristics of the "carrier" particle as well as the organic compound. Various respiratory tract tissues have been shown to metabolize the particle associated compounds, benzo(a)pyrene (BaP) and nitropyrene (NP). Clearance of deposited particles is conveniently characterized in three phases. Early clearance appears to be similar across the species studied. Intermediate-phase clearance rates appear to vary depending on species, dose and duration of exposure. Lung clearance rates for the mouse and rat are approximately 4 times greater than those for humans and dogs. The late phase occurs during impaired removal. Repeated exposure to sufficiently high concentrations results in excessive particulate loading. Particle-laden macrophages exhibit decreased mobility and phagocytic activity resulting in sluggish transport of particles from the lungs. Experiments in the rat have shown that at high exposures the mass of diesel exhaust particles remaining in the lung does not appear to reach a steady state as exposure time increases (Mauderly *et al*., 1987). This overload phenomenon occurs because lung clearance does not keep up with deposition. Normally, clearance rates in the human lung are substantially slower than clearance in rat lungs. This information was considered in interpreting the animal bioassay results and the human epidemiological studies and in extrapolating the results to ambient concentrations. Information on toxicokinetics is used to predict a relationship between exposure and accumulation of diesel soot in the lung.

1.4.2 USING THE RAT STUDY TO PREDICT CANCER RISKS IN HUMANS

Unit cancer risk estimates were derived from rat studies using two different measures of dose. First, the relationship between ambient concentration and lung tumor formation was examined. Second, lung tumors were analyzed as a function of cumulative lung burden over time. The latter dose measure was assumed on theoretical grounds to be a better predictor of tumorigenicity.

The results of Mauderly *et al.*, using two different models of carcinogenicity and the two measures of exposure, yield a lowest calculated 95% UCL for unit risk of 0.5×10^{-4} $(\mu g/m^3)^{-1}$. This value is based on the Moolgavkar cell-proliferation model using rat lung burden extrapolated to human lung burden per alveolar surface area. The highest 95% UCL for unit risk value from the Mauderly *et al*. study was 3×10^{-4} $(\mu g/m^3)^{-1}$ using the Weibull model with rat inhalation exposure levels as input, and extrapolation based on intake per body surface area.

While the data of Mauderly *et al*. (1987) were determined to be most applicable among rat data sets to estimate cancer potency, a comparative evaluation of several multiple dose experiments with rats was also conducted using the quantal form of the linearized multistage (LMS) model. This model has been extensively used by OEHHA, U.S.EPA, and other public health organizations (DHS, 1985; U.S.EPA, 1986; WHO, 1996) and the application here was developed in consultation with the U.S.EPA. Among the five rat studies, after fitting the rat data and scaling the results to humans using a 70 year lifetime, the lowest 95% UCL for risk was 1×10^{-5} $(\mu g/m^3)^{-1}$, and the highest value was 2×10^{-4} $(\mu g/m^3)^{-1}$. In their 1994 and 1998 draft risk

assessment, the U.S.EPA applied the linearized multistage model to the three of these five animal bioassays then available to derive human unit risk estimates. Their analysis provided similar unit risks.

The uncertainty in the application of these rat predictions to humans is substantial. The scaling of such important characteristics as clearance rates, the presence or absence of a threshold for onset of carcinogenic effects, or the possible presence of multiple carcinogenic mechanisms all contribute to the uncertainty. The present lack of knowledge about how the carbon core of the diesel exhaust particle contributes to carcinogenicity also adds to the uncertainty about the scaling from rats to humans. For example, it has been suggested that rodent lung tumor induction by particles such as carbon black and titanium dioxide may not be relevant to human cancer risk if due to an overwhelming of particle clearance and no other tumor responses are noted (Commission on Risk Assessment and Risk Management, 1996). Thus, the human risk estimates, based upon the rat data alone, are especially uncertain and may be substantially higher or lower.

Due to the above uncertainties and the availability of epidemiological data, OEHHA has placed the quantitative risk assessment based on rat studies in Appendix G and has focused on the risk estimates from epidemiological data for the final range of unit risks for humans.

1.4.3 QUANTITATIVE RISK ASSESSMENT BASED UPON OCCUPATIONAL STUDIES

The Garshick *et al*. (1987a) case-control study and the Garshick *et al*. (1988) cohort study of U.S. railroad workers were used to estimate the risk of lung cancer in the general population due to diesel exhaust. These two studies were selected for quantitative risk assessment because of their quality, their apparent finding of a relationship of cancer rate to duration of exposure, and the availability of measurements of diesel exhaust among similar railroad workers from the early 1980's in other studies. The case-control study (1987) has an advantage in providing direct information on smoking rates, while the cohort study (1988) has an advantage of smaller confidence intervals in the risk estimates.

The relative risks reported in these studies were related to estimates of the actual exposures to develop a quantitative risk assessment. In order to bracket the exposure of the railroad workers to diesel exhaust a variety of patterns of exposure are considered. The patterns are characterized by two components: a) the extent of change from 1959 to 1980 in diesel exhaust exposure, expressed as a ratio, and b) the average exposure concentration for the workers on trains measured in the Woskie *et al*. (1988a) study, the baseline. The alternative ratios are as follows: a) a ratio of 1 suggested and used in Crump *et al*. (1991) as more realistic than the Garshick *et al*. (1987a, 1988) assumption of constant concentration from 1959-1980 and none before that; b) a ratio of 2 suggested by K. Hammond (1998) to allow for a modest peak in 1959; c) a ratio of 3 allowing for more peak, a scaled down version of the exposure factor of 10 that Woskie *et al*. (1988b) reported for exposure concentration of shopworkers to nitrogen dioxide in enclosures including engine test sheds; and d) a ratio of 10, peak of the magnitude of values for the engine test sheds. The alternative baselines are as follows: 40, obtained by subtracting the background

measurement of the unexposed workers from the measurement of the train workers, rounded down (see Table 7-9 footnotes: 82-39=43); 50, same but rounded up to allow somewhat for measurements of workers on trains not having as much non-diesel exhaust background as the clerks; and 80, obtained by assuming that the entire ETS-adjusted RSP of the train workers is diesel exhaust while the clerks are considered unexposed to diesel exhaust (0 concentration).

1.4.3.1 THE GARSHICK (1987A) CASE-CONTROL STUDY

For the Garshick (1987a) case-control study, OEHHA relied upon the exposure reconstructions described above to develop a range of unit risk values. The highest 95% upper confidence limit (UCL) value derived for this study was 2.4×10^{-3} $(\mu g/m^3)^{-1}$; this value was based upon the assumption of a ramp pattern and a 50 $\mu g/m^3$ exposure level. The lowest 95% UCL value of 3.6×10^{-4} $(\mu g/m^3)^{-1}$ is based upon the roof pattern of exposure with the highest exposure 10-fold above the 50 $\mu g/m^3$ exposure level. Unit risk estimates based on different exposure reconstructions and the data in Garshick (1987a) are presented in Table 1-1.

1.4.3.2 ANALYSES OF THE GARSHICK *ET AL*. (1988) COHORT

Estimates of unit risk based on the retrospective cohort study of U.S. railroad workers by Garshick *et al.* (1988) also relied on reconstructions of exposure. The highest 95% upper confidence limit (UCL) value derived using the cohort study was 1.8×10^{-3} $(\mu g/m^3)^{-1}$ based on the assumption of a ramp pattern and 50 $\mu g/m^3$ exposure level. The lowest 95% UCL value using biologically-based modeling of 1.3×10^{-4} $(\mu g/m^3)^{-1}$ is based upon the roof pattern of exposure with the highest exposure 3-fold above the 50 $\mu g/m^3$ exposure level.

The Garshick *et al*. (1988) cohort study has been identified as being among the most comprehensive and extensive epidemiological data sets with regards to the carcinogenic effects of diesel exhaust exposure. Even so, due to limitations in the data set, and of epidemiological studies in general, a number of questions have been raised as to the application of this data set to the quantitative risk assessment of diesel exhaust. One of the key issues with regard to the cohort is whether or not the data show dose-response. The original publication indicated that among this cohort there was a small but significantly elevated risk for lung cancer. In December 1994, U.S.EPA released a comprehensive health review of diesel exhaust. This draft document included a reanalysis (Crump *et al*., 1991) of the dose response of Garshick *et al.* (1988) cohort study. While the reanalysis appeared to show the absence of a dose-response relationship, subsequent reanalyses by OEHHA continued to find evidence of a dose-response relationship (Appendix D).

In January, 1996, the Health Effects Institute (HEI) and OEHHA organized a joint workshop with the Air Resources Board, U.S.EPA, the World Health Organization (WHO), and the National Institute for Occupational Safety and Health (NIOSH) to bring together recognized experts and interested parties to help clarify and resolve differences in analyzing and interpreting the epidemiological data on diesel exhaust, particularly the Garshick *et al.* (1988) cohort study. Since that time, OEHHA has continued an open scientific exchange regarding ways to better assess the Garshick data. While there still appears to be differences in the scientific

interpretation of the Garshick data, the differences in assumptions, approaches and results are now clearer and better understood.

Appendices E and F summarize the nature of and bases for these differences. Appendix D provides further analyses of the cohort data in Garshick *et al*. (1988).

A meta-analysis (Appendix C) of the epidemiological studies found that the Garshick cohort study reported a relative risk which is reasonably consistent with the range of lung cancer risks associated with diesel exhaust exposure in other studies. Further analyses of the Garshick cohort (Chapter 7, Appendix D) based upon exposure reconstructions provide a number of positive 95% UCL risk estimates.

In addition to a quantitative risk assessment utilizing the Garshick *et al*. (1988) cohort study, our risk assessment includes estimates based upon the results of the Garshick *et al*. (1987a) case-control study. Such estimates are consistent with the results of the meta-analysis and the similar conclusion by HEI of a small but consistent elevated risk of lung cancer in the occupational studies.

Calculations presented in Chapter 7 and the reanalyses of the individual data of the Garshick *et al*. (1988) cohort study in Appendix D provide a number of estimates of unit risk, with the range of 95% UCL described in Table 1.1.

1.5 SOURCES OF UNCERTAINTY IN QUANTITATIVE RISK ASSESSMENT

Results based on the human data and those based on the animal data are both subject to considerable uncertainty. The strengths and weaknesses of calculating population risks using the human studies (Garshick *et al*., 1987a; Garshick *et al*., 1988) and the animal bioassay (Mauderly *et al*. 1987a; Brightwell *et al*., 1989; Heinrich *et al*., 1995; Ishinishi *et al*., 1986a; Nikula *et al*., 1995) are summarized in Table 7-6.

The principal uncertainties in using the rat data are their application to humans in terms of response, the choice of dose-response model to extrapolate the risk to environmental concentrations, and the range of dose extrapolation involved.

The principal uncertainties in using the human data are the representativeness of railroad workers for the general population, the choice of the analytical model, and the lack of knowledge of the exposure history of the railroad workers including possible exposure to unknown confounders. The historical reconstruction here is based upon the Woskie *et al*. (1988b) exposure data for railway workers and the rate of dieselization for U.S. railroads. Using a range of reduced emission assumptions, alternative exposure patterns are considered. This reconstruction takes into account to some degree the likely higher exposure levels in the past. If actual exposures were higher than assumed here, then our estimates of the risk would be lower. And if exposures were lower, then the estimated risks would be higher. The range of extrapolation from these estimated occupational exposure levels to the California population-weighted annual average exposure of 1.54 µg diesel exhaust particulate/m^3 is not large.

The presence or absence of a dose-response threshold is another source of uncertainty. The *in vitro* and *in vivo* genotoxicity of diesel exhaust suggests that a non-threshold mechanism for carcinogenesis may be involved. The Moolgavkar quantitative analyses of the rat cancer bioassay did not suggest there was a threshold for the carcinogenicity of diesel exhaust in the rat. In addition, as discussed in Appendix B, epidemiological studies have observed increases in the relative risk for lung cancer in association with exposures of the general population to ambient particulate matter. On the other hand, evidence that diesel exhaust particulate matter at high concentrations exceeds pulmonary clearance capabilities and causes chronic inflammation so as to increase production of inflammatory cytokines and cell proliferation may suggest the presence of a threshold. However, at present, the limited evidence available does not allow a threshold value for carcinogenesis to be identified.

On balance, the human data lend more confidence in the prediction of human risks than the data from the rat studies because of the uncertainties of extrapolating from rats to humans, especially in the context of a substantial particle effect. The uncertainties of extrapolating from rats to humans appear to outweigh the uncertainties of using the epidemiological results, namely, the uncertainties of the actual exposure history, modeling, and data selection. The exposure reconstructions bracket the overall exposure and therefore they bracket the risk. The uncertainty in the extrapolation from animal data is difficult to quantify, but is likely to be much greater. Extrapolations of either the animal or human data involve additional sources of uncertainty with respect to both model and data selection.

A number of individuals and organizations have indicated that the epidemiological studies are limited in their application to environmental risk assessment. OEHHA recognizes that the limited exposure information available does contribute to the overall uncertainty of the dose response risk assessment for diesel exhaust based upon the epidemiological findings. However, the overall magnitude of the associated uncertainty is not unduly large. The greater than unusual uncertainty in the exposure estimates is substantially offset by the much smaller than usual range of extrapolation from the occupational exposures of interest to the ambient levels of concern here. The availability of human data obviates the need to use animal data thus avoiding uncertainties of animal-to-human extrapolation. OEHHA provided a tabular range of risk so as to fairly capture the scope of the uncertainty in these analyses.

1.6 CONCLUSIONS AND RANGE OF RISK ESTIMATES

A reasonable and likely explanation for the increased rates of lung cancer observed in the epidemiologic studies is a causal association of diesel exhaust exposure with lung cancer. OEHHA therefore estimated the magnitude of the cancer risks associated with the range of human exposures. OEHHA has provided a range of values to describe the magnitude of the potential risks.

Based on the human data, the principal finding of this quantitative risk assessment is a range of lifetime unit risk (95% UCL) as shown in the right-hand column of Table 1-1; with the lowest risk estimate of 1.3×10^{-4} (lifetime - $\mu g/m^3)^{-1}$ and the highest risk estimate of 2.4×10^{-3}

(lifetime - $\mu g/m^3)^{-1}$. The geometric mean unit risk obtained from the end points of the range of values in Table 1-1 is 6×10^{-4} (lifetime - $\mu g/m^3)^{-1}$. The geometric mean provides information on the central tendency of the range and is not to be confused with a best estimate identified from the available calculations. The lower end of the range is the rounded value for both forms of multistage model using the roof exposure pattern for the data of the Garshick *et al*. (1988) cohort study of U.S. railroad workers. OEHHA concludes that the more scientifically valid unit risk values are near the lower end of the range based on analyses conducted in Chapter 7 and Appendix D.

The values based on rat data are slightly within or below the bottom of the range of values based on human data. This divergence may be due to a greater sensitivity of humans, to underestimates of exposure in the human studies, to lack of knowledge of the appropriate way in which to calculate scaling from rodents to humans or to other factors. For instance, the wide range in risk values obtained using human data and those obtained using rat data may reflect the substantially greater background rate of lung cancer in humans (due to smoking). As described in Appendix G, the range of 95% UCL for risk from the five rat studies, after fitting the rat data and scaling the results to humans, is 1×10^{-5} to 3×10^{-4} $(\mu g/m^3)^{-1}$. A geometric mean unit risk estimate from the rat studies determined in this document and in U.S. EPA (1994) is 6×10^{-5} (lifetime-$\mu g/m^3)^{-1}$.

The uncertainty in extrapolating the rat findings to humans is substantial. The scaling of such important characteristics as clearance rates, the presence or absence of a threshold for onset of carcinogenic effects, or the possible presence of multiple carcinogenic mechanisms all contribute to the uncertainty. The present lack of knowledge about how the carbon core of the diesel exhaust particle contributes to carcinogenicity also adds to the uncertainty about the scaling from rats to humans. Because of these uncertainties and the availability of epidemiological data, OEHHA has decided to use risk estimates based on human data for the final range of risks to humans (see Table 7-6).

The strengths and weaknesses of calculating population risks using the human studies (Garshick *et al*. 1988, Garshick 1987 *et al*.) and the animal bioassays (Mauderly *et al*., 1987; Brightwell *et al*., 1989; Heinrich *et al*., 1995; Ishinishi *et al*., 1986a; Nikula *et al*., 1995) are presented in Table 7-6. An approximate correction for smoking would raise the rat-based unit risks into near coincidence with the human-based unit risks as applied to the California population. On balance, the human data lend more confidence in the prediction of human risks than the data from the rat studies because of the uncertainties of extrapolating from rats to humans, especially in the context of a substantial particle effect.

Table 1.1 presents the 95% UCL lifetime unit risks per $\mu g/m^3$. The Air Resources Board has estimated that the average annual ambient concentration of diesel exhaust to which Californians are exposed is 1.54 $\mu g/m^3$; this includes both indoor and outdoor exposure. The upper limit of potential additional cancer cases *over a lifetime* in California can be estimated using the cancer unit risk values in Table 1-1 and the Air Resources Board estimate of the average ambient concentration of diesel exhaust to which Californians are exposed. This estimate is a range from 200 to 3600 additional cancer cases for every one million Californians over a 70 year lifetime.

The estimate was derived by multiplying the average statewide concentration of diesel exhaust (1.54 $\mu g/m^3$) times the highest and lowest cancer unit risk values found in Table 1-1 and rounding to one significant digit. OEHHA concludes, based on analyses presented in the Technical Support Document, that the more scientifically valid unit risk values and subsequent estimates of the upper limit of potential additional cancer cases are near the lower end of the ranges.

Based upon its own analysis and that of the U.S.EPA, OEHHA recommends the adoption of 5 $\mu g/m^3$ (the 1993 U.S.EPA RfC) as the chronic inhalation Reference Exposure Level (REL).

Based upon the cancer risk to the public and the potential short-term and long-term respiratory effects of diesel exhaust, diesel exhaust appears to meet the definition of a toxic air contaminant (Health and Safety Code Section 39655). Diesel exhaust is "an air pollutant which may cause or contribute to an increase in mortality or in serious illness, or which may pose a present or potential hazard to human health."

Table 1-1. Summary of Cancer Unit Risks According to Study, Exposure Assumptions, and Modeling Approaches.

Garshick *et al*. (1987a) Case Control[1]	95% UCL Cancer Unit Risk $(\mu g/m^3)^{-1}$
Scenario[2]	
A	2.4×10^{-3}
B	1.8×10^{-3}
C	1.0×10^{-3}
D	6.6×10^{-4}
E	3.6×10^{-4}
Garshick *et al*. (1988) Cohort Study (Chapter 7)[3]	
Scenario	
A	1.8×10^{-3}
B	1.4×10^{-3}
C	8.2×10^{-4}
D	5.1×10^{-4}
E	2.8×10^{-4}
Garshick *et al*. (1988) Cohort Study Appendix D)[4]	
Scenario A	
general multiplicative model	1.9×10^{-3}
biologically based[5]	3.8×10^{-4}
Scenario C	
general multiplicative model	7.2×10^{-4}
biologically based [5]	1.3×10^{-4}
biologically based [6]	1.5×10^{-4}

[1] Using published slope coefficient for hazard on years to diesel exhaust as described in 7.3.3.

[2] A Ramp pattern of exposure plateauing in 1959 at the 1980 exposure level of 50 $\mu g/m^3$.
B Roof pattern of exposure peaking in 1959 at twice the 1980 exposure level of 40 $\mu g/m^3$.
C Roof pattern of exposure peaking in 1959 at 3-fold the 1980 exposure level of 50 $\mu g/m^3$.
D Roof pattern of exposure peaking in 1959 at 3-fold the 1980 exposure level of 80 $\mu g/m^3$.
E Roof pattern of exposure peaking in 1959 at 10-fold the 1980 exposure level of 50 $\mu g/m^3$.

[3] Using individual data to obtain a slope for hazard on years of exposure to diesel exhaust as described in Section 7.3.4.

[4] Applying time varying concentrations to individual data to obtain a slope of hazard on exposure as described in Appendix D.

[5] $6^{th}/7$ stage model.

[6] $7^{th}/7$ stage model.

2.0 INTRODUCTION

This health risk assessment for diesel exhaust provides the health evaluation and recommendations constituting the basis for the Air Resources Board (ARB) of the State of California to identify diesel exhaust as a toxic air contaminant, pursuant to Health and Safety Code Section 39660. This present document is the third draft document prepared in response to an October 2, 1989 memorandum from the ARB to the Department of Health Services. This memorandum requested evaluation of the health effects of diesel exhaust as a candidate toxic air contaminant.

In June 1994, March 1997, and February 1998 the Office of Environmental Health Hazard Assessment (OEHHA) released draft documents, *Health Risk Assessment for Diesel Exhaust*, for public comment. The Scientific Review Panel reviewed the document at their meeting in October 1997, held a meeting with invited scientists in March 1998, and held a final meeting where they approved the document on April 22, 1998. The present document has been updated in response to ipublic comments and comments by the Scientific Review Panel.

There has been much collaboration and consultation in the development of the OEHHA documents on diesel exhaust. With respect to the preparation of the first draft, OEHHA participated in an ARB conference, "Risk Assessment of Diesel Exhaust: 1990 and Beyond," to discuss exposure and health issues related to diesel exhaust with national experts, including individuals from the United States Environmental Protection Agency, the University of California and the engine manufacturers. In June, 1992, a joint ARB-OEHHA-UCB paper (Denton *et al.* 1992) at the Air and Waste Management Association annual meeting presented a summary of initial findings of the review for the present document. Upon the release of the draft document, OEHHA held two public workshops for the purposes of explaining and soliciting input from the public and members of the scientific community. In addition, the ARB and OEHHA received over 1,000 pages of public comments regarding the previous drafts which have been considered in the preparation of Part B of this draft. *Part C, Response to Public Comments*, provides our formal responses to the comments received on the last draft (February 1998).

After the release of the 1994 draft document, the U.S.EPA in November of 1994 and the HEI in May of 1995 each released comprehensive risk assessment documents on diesel exhaust. These documents differed from each other and our 1994 draft especially with respect to the use and interpretation of the available animal and human health hazard information relating to the carcinogenicity of diesel exhaust. In early 1996, the OEHHA, HEI, ARB, WHO, and NIOSH cosponsored an international workshop to discuss the bases of the scientific differences of opinion regarding the carcinogenic potential of diesel exhaust. This workshop focused upon the use of the available human epidemiological information to estimate the potential cancer risk to persons exposed to diesel exhaust. This two-day workshop particularly concerned itself with the analysis of the dose-response trend in Garshick cohort study of railway workers (Garshick *et al.* 1988). This document reflects the benefit of those discussions. In particular, Appendix C was developed to quantify the overall conclusions and graphical presentation in the epidemiology analysis of the HEI report. Furthermore, Appendices E and F were added to help clarify the underlying scientific issues in specific quantitative analyses of the available data.

Following this chapter are the sections of the text, tables, and figures that support the summary of Chapter 1.

Chapter 3, *Toxicokinetics*, reviews the literature of the transport of diesel exhaust into and out of the lung. The text includes consideration of the tendency of the particulate phase to be retained in the lung after inhalation of diesel exhaust at high exposure concentrations.

Chapter 4, *Noncarcinogenic Effects*, reviews the literature on those adverse health effects that are not specifically related to genotoxicity or carcinogenicity. The document includes discussion of the recent immunotoxic effects reported for both humans and animals. The primary result from this section is the designation of a reference concentration (RfC) in agreement with that of the U.S.EPA. The U.S.EPA's IRIS document supporting their RfC is incorporated as Appendix A, Reference Concentration for Chronic Inhalation Exposure. This section also refers to the evidence for health effects of respirable particulate matter by reference to Appendix B, Health Effects of Ambient Particulate Matter.

Chapter 5, *Genotoxicity*, has information on the results of genotoxicity tests, discussion of bioavailability under physiological conditions, information on DNA adducts in humans and animals exposed to diesel exhaust, and information on induction of oxidative DNA damage by intratracheal instillation of diesel exhaust particulate matter.

Chapter 6, *Carcinogenic Effects*, has information on diesel exhaust and diesel exhaust particulate matter carcinogenicity bioassays, a discussion of potential mechanisms of action of diesel exhaust-induced rat lung tumor induction, information from the meta-analysis (see below), and extensive discussion of several issues in the causal inference subsection.

Appendix C, *Quantitative Meta-analysis on the Relationship of Occupational Exposure to Diesel Exhaust and Lung Cancer*, assesses the consistency in results among the epidemiological studies with respect to the potential of diesel exhaust exposure to cause lung cancer. The results of this appendix are important to the overall qualitative conclusion reached in Chapter 6.

Chapter 7, *Quantitative Cancer Risk Assessment*, describes the analysis of the epidemiological data to estimate human cancer risk. A number of numerical corrections suggested by the public comments were made. The document now bases the range of unit risk estimates only on the epidemiologic information. We evaluated the exposure data for the railroad workers and have identified a range of exposures and exposure patterns for use in the quantitative risk assessment.

Appendix D presents calculations of the relationship of risk to diesel exhaust exposure, using the individual data in the Garshick *et al*. (1988) cohort study of US railroad workers. The analyses use different models and time patterns of exposure to explore the influence on calculated risks.

Appendix E, *Differences in Approach to Analyzing the Garshick et al. (1988) Cohort*, focus on issues related to the validity of the dose-response relationships obtained from that study.

Appendix F, *Effect of Model Assumptions on Exposure-Risk Relationship for the Garshick et al. (1988) Cohort Study*, has been added to the document and it further evaluates dose-response related issues.

Appendix G, presents the quantitative risk assessment based on the rat bioassay data.

3.0 TOXICOKINETICS

The emissions from diesel engines contain both gaseous and particulate constituents. The gaseous constituents include carbon dioxide, carbon monoxide, nitric oxide, nitrogen dioxide, oxides of sulfur, and hydrocarbons (e.g. ethylene, formaldehyde, methane, benzene, phenol, 1,3-butadiene, acrolein and some polynuclear aromatic hydrocarbons (PAHs)and nitro-PAHs. Particles in diesel exhaust have solid carbon cores that are produced during the combustion process and tend to form chain or cluster aggregates. More than 95% of these particles are less than 1 μm in size. Estimates indicate that as many as 18,000 different substances from the combustion process can be adsorbed onto diesel exhaust particles. The adsorbed material constitutes 15 to 65% of the total particulate mass and includes such organic compounds as PAHs and nitro-PAHs (Cuddihy *et al*., 1984). The organic compounds adsorbed on diesel exhaust particles are of interest because they include mutagens and carcinogens (See Sections 5 and 6). Which organic pollutants are carried on a particle, how much, and how they later become separated from the particle are, in part, affected by physical and chemical properties of both the organic pollutant and the "carrier" particle (Sun *et al*., 1988).

The biological fate of the inhaled particle-associated compounds is influenced by distribution, clearance, and metabolism in the body, particularly in the lung. In turn each of these parameters can be influenced by the characteristics of the "carrier" particle as well as the organic compound. Various respiratory tract tissues have been shown to metabolize the particle associated compounds, benzo(a)pyrene (BaP) and nitropyrene (NP). Clearance of deposited particles is conveniently characterized in three phases. Early clearance appears to be similar across the species studied. Intermediate-phase clearance rates appear to vary depending on species, dose and duration of exposure. Lung clearance rates for the mouse and rat are approximately 4 times greater than those for humans and dogs. The late phase occurs during impaired removal. Repeated exposure to sufficiently high concentrations results in excessive particulate loading. Particle-laden macrophages exhibit decreased mobility and phagocytic activity resulting in sluggish transport of particles from the lungs. Experiments in the rat have shown that at high exposures the mass of diesel exhaust particles remaining in the lung does not appear to reach a steady state as exposure time increases (Mauderly *et al*., 1987a). This overload phenomenon occurs because lung clearance does not keep up with deposition. This section will discuss the deposition of diesel exhaust particulate and associated organic compounds, clearance from the lung, lung metabolism and distribution of various components throughout the body.

3.1 LUNG DEPOSITION

Based upon differences in physiological function and particle clearance characteristics, the respiratory tract has been subdivided into three regions (Task Group on Lung Dynamics, 1966): 1) naso- or oro-pharyngeal; 2) tracheobronchial; and 3) alveolar. The naso- or oro-pharyngeal region extends from the anterior nares or lips to the larynx. The tracheobronchial region covers the airways from the trachea to terminal bronchioles, inclusively. These first two regions of the respiratory tract are ciliated and lined by mucus. The airways beyond the terminal bronchioleare the alveolar region of the lungs, where gas exchange takes place. These airways are nonciliated and are lined with surfactant.

There are four mechanisms which cause deposition of particles within the respiratory tract (Yu and Xu, 1987): impaction, sedimentation, interception, and diffusion. The contribution from each individual mechanism to total deposition depends upon the particle size and flow rate. Under normal breathing conditions, small particles, < 0.5 μm, are deposited mainly through diffusion. Because of their small size, < 0.2 μm, most diesel particles deposit through diffusion, and the role of the other mechanisms is minor (Yu and Xu, 1987).

The deposition of diesel particles or surrogate diesel particles (i.e. inert particles of similar size, such as Ga_2O_3) has been studied in rats, guinea pigs and dogs (Chan *et al*., 1981; Lee *et al*., 1983; Strom *et al*., 1989; Wolff *et al*., 1987; Brooks *et al*., 1981) utilizing radiolabeled particles. Deposition of diesel exhaust in humans has been mathematically modeled by Yu and Xu (1987). The results of these studies are summarized in Table 3-1. The pulmonary deposition efficiency -- mass deposited as a percent of mass inhaled -- appears to be fairly similar across the species studied thus far. In general, the efficiency of pulmonary deposition falls in the range of 12 to 20%.

In their model studies, Yu and Xu (1987) estimated a 10 to 13% efficiency of alveolar deposition for diesel particles size 0.1 to 0.3 μm (geometric standard deviation 4.5) in adult humans under normal breathing conditions. Breathing mode, whether by nose or mouth, did not appear to affect efficiency of alveolar deposition. However, at a given respiratory frequency, increasing the minute ventilation (i.e. the number of breaths per minute multiplied by the tidal volume) increased the efficiency of alveolar deposition significantly.

Total and regional deposition efficiencies were also evaluated as a function of age. Both total and regional deposition of diesel particles were higher in children above 2 years of age than in adults. Although head and tracheobronchial deposition decreased monotonically as age increased, alveolar deposition peaked at about five years of age. Total deposition reached a maximum at the age of two years.

Yu and Xu (1987) also compared the measured deposition efficiencies for laboratory species to the efficiencies predicted by their model. Predicted values agreed well with actual data. Despite the fact that the body weight of humans and laboratory animals differ by several orders of magnitude, the difference between deposition efficiency predicted for humans is within 30% of deposition efficiency measured in laboratory animals. An increase in body weight across species was associated with a slight decrease in deposition efficiency.

3.2 LUNG CLEARANCE

The sections on clearance consider results for acute exposure, for chronic and subchronic exposure and for clearance of particle-associated organics. Lung clearance of particles is an important defense mechanism against inhaled particulate matter. The clearance of particles has

an early, an intermediate phase and a late phase (Wolff *et al*., 1987). The early phase characterizes the rapid removal of particles deposited in the tracheobronchial tree or in the proximal respiratory bronchioles via the mucociliary escalator. The intermediate phase characterizes the slow removal of particulate matter from the pulmonary region by processes which may involve endocytosis, absorption, dissolution and metabolism of the particles. The late phase refers to the impaired clearance due to overloading of macrophages by particles, leading to sequestration of those macrophages in the lung alveoli. Under normal conditions, most of the particles deposited in the pulmonary region are first engulfed by alveolar macrophages which are then cleared by transport to the bronchial airways or the lymphatic system. In repeated high exposures, clearance is seriously impaired, and the late phase needs to be considered.

Alveolar clearance rates for non-diesel particles vary markedly between species, and are considerably longer in dogs and humans, compared with rodents (Snipes, 1989a,b). In addition, previous lung burdens of particulates slow the alveolar clearance process (Morrow *et al*., 1991). Strom *et al*. (1990) found that low concentrations of particles given over a long period of time resulted in greater retention in the lung than high concentrations over a short time, thus complicating the issue of extrapolating from short-lived rodents to human data.

3.2.1 RESULTS FOR ACUTE EXPOSURES

Two of the lung clearance studies conducted to date with acute exposures have utilized the rat as the animal model (Chan *et al*., 1981; Lee *et al*., 1983). This study also utilized guinea pigs (Lee *et al*., 1983). Lung clearance studies are summarized for acute exposures in Table 3.2. The time course of the early phase (tracheobronchial clearance) appears to be similar across the two species studied and independent of dose or exposure duration.

Intermediate clearance rates, however, appear to depend on the dose, the exposure duration and the species examined. Acute exposure protocols do not appear to disrupt normal alveolar clearance; so the acute studies give baseline intermediate-phase clearance half-times in rats, ranging from approximately 40 to 80 days with no statistical difference between control and exposed groups (see Table 3.2). Guinea pigs appear to be essentially unable to clear diesel exhaust particles deposited in the alveolar region (Lee *et al*., 1983). Following acute exposure to ^{14}C-tagged diesel exhaust (7 mg/m^3 for 45 min.), very little alveolar clearance was observed from day 10 to 432 postexposure. Only early phase (tracheobronchial) clearance was observed.

Snipes *et al*. (1983) conducted a study to compare retention of ^{134}Cs-labeled fused aluminosilicate particles (FAP) inhaled by three animal species: dog, rat and mouse. The study period in this investigation was quite long, up to 850 days (nearly 28 months) after exposure to ^{134}Cs-FAP. Dogs, rats and mice were briefly (15 - 50 min) exposed to 0, 1.5 or 2.8 μm particles at concentrations ranging from 1 to 100 mg/m^3. Compared to dogs, the rats and mice demonstrated a rapid clearance from the pulmonary region, mainly due to the clearance of particles to the gastrointestinal tract. The dogs cleared deposited particles at a slower rate, with most of the long-term clearance going to the lung-associated lymph nodes (LALN). The long-

term overall alveolar clearance half-times were approximately 460, 690 and 2300 days in mice, rats and dogs, respectively. The pulmonary clearance rate in dogs was 3.3 to 5 times slower than in rats and mice. The authors refer to evidence of retention and deposition patterns in humans being close to those in dogs but not to those in rats or mice. Clearance to LALNs is also known to occur in humans; however, the fraction of deposited pulmonary particles cleared to this compartment is not known.

Bohning *et al*. (1982) and Bailey *et al*. (1982) estimated clearance of larger particles (1.2 to 3.9 μm) in humans after the first day following acute exposure. Normal lung clearance was found to occur at two rates: one with a half-time of 20 to 30 days and the other with a half-time of 300 to 420 days. Approximately 60 to 88% of the retained particles were cleared via the slow phase. Bohning *et al*. (1982) also investigated lung clearance of larger particles (1.2 and 3.6 μm) in individuals exhibiting impaired lung clearance. Twenty-five volunteers were evaluated: 5 healthy non-smokers; 6 healthy ex-smokers; 8 smokers; and 6 individuals with chronic obstructive lung disease (COLD). The healthy non-smokers and ex-smokers exhibited normal clearance rates, one with a half time of 30 $\pm$ 23 days and one with a half time of 296 $\pm$ 98 days. Smokers exhibited a suppression of the more rapid clearance rate and increased the half-time of the slower clearance rate by 14.7 $\pm$ 3.0 days per pack-year of smoking. Individuals with COLD exhibited significantly larger clearance half-times (660 $\pm$ 432 days) for the slower clearance rate.

3.2.2 EFFECT OF CHRONIC EXPOSURE

Accumulated lung burdens of particles chronically inhaled represent a balance between the deposited particles minus those cleared (Wolff *et al*., 1986). The percent retention following high levels of exposure to diesel exhaust (> 3.5 mg/m^3) is 2 to 3 fold greater than following low level exposures (< 1 mg/m^3). This observation suggests that at higher exposure concentrations, alterations in either deposition or clearance of diesel particles occurs. Based on the available data, impaired clearance appears to be predominantly responsible since deposition rates do not change significantly with dose level (Wolff *et al*., 1987 see Table 3.1).

Repeated exposure to sufficiently high concentrations of particles appears to result in excessive particulate loading of the macrophages. Particle-laden macrophages lose some of their mobility and phagocytic activity, resulting in sluggish transport of particles from the lungs (Lee *et al*., 1987). In experimental animals, when the ability of alveolar macrophages to engulf and remove particles from the lung is exceeded by the continued deposition of particles, the macrophage lung clearance mechanisms are considered to be overloaded. At this stage, further increases in lung burden do not lead to an increase in clearance. Ballew *et al*. (1995) examined an epidemiological study by Smith *et al*. (1984) of 170 workers exposed to working conditions containing silicon carbide particulates. Using goodness-of-fit as a guide for choosing an appropriate model, these authors found that the data implied that a slow rate of clearance rather than an overload occurred in these workers. However, differences in goodness of fit between alternative models was small, so the evidence does not definitively show an absence of an overload effect in these workers. Several groups of investigators have examined the effects of repeated particle exposure on lung clearance (Griffis *et al*., 1983; Chan *et al*., 1984; Heinrich *et al*., 1986a; Wolff *et al*., 1986, 1987;

Lee *et al*., 1987; Lewis, 1986; Strom, 1989; Strom *et al*., 1988). Oberdorster (1995) reported that lung clearance is impaired in experimental animals when the particle burden reaches a volume of 1 μL/g of lung. Two informative studies will be discussed in detail in the following paragraph (Chan *et al*., 1984; Wolff *et al*., 1987). Those studies and the remaining chronic studies are summarized in Table 3.3.

Chan *et al*. (1984) conducted an extensive study of the effects of prolonged exposure to diesel exhaust particles on pulmonary retention. Male Fischer 344 rats were first exposed to clean air, 0.25 or 6 mg/m^3 diesel exhaust for 20 hours/day, 7 days/week for periods varying from 7 to 112 days, followed by a nose-only exposure to ^{14}C-tagged diesel particles for 45 min. At preselected time intervals up to 1 year after the radiolabel exposure, the ^{14}C-activity in the lung was measured to estimate lung retention. The lung retention data, expressed as a percentage of the initial lung deposition, are an indirect measurement of the overall clearance of particles from the lungs. The pulmonary retention was greater in animals which had been pre-exposed to diesel exhaust. This was described using a late phase residual component in the lung retention model. The late phase was attributed to sequestered macrophage aggregates having essentially so little mobility that their clearance half-times would exceed the normal lifespan of the animals. The late phase was estimated to represent 7, 13.3, 18, 45.5 and 88% of the initial ^{14}C-tagged particle deposition for animals with lung burdens of 0.2, 0.5, 0.7, 6.5 and 11.8 mg, respectively. The proportion of particles retained in the late phase appeared to depend on the lung particle burden in an approximately linear manner. In other words, residual lung retention increased linearly with increasing lung particle burden.

With lung burdens less than 0.7 mg a component of alveolar clearance occurred at nearly the normal rate. In essence, this “normal” clearance was maintained by a sufficient number of clean macrophages at the early stages of the particle sequestering process. As the number and size of the macrophage aggregates increase, reduced mobility was apparent with more active macrophages becoming attached to the aggregates and becoming immobilized. At a lung burden of 6.5 mg, increased lung retention was attributed to both the sequestering of particles by aggregated macrophages and a significantly slower active alveolar clearance. At the highest lung burden, 11.8 mg, normal particle transport mechanisms were apparently eliminated.

The lung burden of 0.7 mg was produced by exposure to 6 mg/m^3 for 20 hours/day for only 7 days. Exposure to 0.25 mg/m^3, 20 hours/day for 112 days (i.e. 16 weeks) produced lung burdens of 0.5 mg and resulted in a 7% residual component. It is possible that under continuous, chronic exposure even lower air levels could produce similar lung burdens.

Several years later Wolff *et al*. (1986, 1987) conducted detailed studies which also evaluated the effect of chronic exposure on lung burden and lung clearance. The exposure durations were conducted for a much longer period of time than in the study of Chan *et al*. (1984) (i.e. 720 - 900 days vs 7 - 112 days). Wolff and co-workers exposed Fischer 344 rats to clean air, 0.35, 3.5 or 7.0 mg/m^3 diesel exhaust 7 hours/day, 5 days/week for 30 months. Particle clearance was evaluated using three different measurements: (1) Tracheal mucociliary clearance was measured as an indicator of effects on rapid clearance (half-time in hours) from the ciliated airways.

Tracheal mucociliary clearance measurements were made 3 weeks prior to and at 6, 12, 18, 24, and 30 months of exposure using ^{99m}Tc-macroaggregated albumin which was instilled onto the epithelium of the lower trachea. The percentage of material retained at the original instillation site at 60 min was calculated to provide an indicator of tracheal clearance. (2) Clearance of inhaled particles with a short-lived radiolabel (^{67}Ga) was used to estimate the normal long-term clearance (intermediate phase with a half-time in days to weeks). Radioactivity levels from ^{67}Ga were measured at 0.5 hr and 1, 4, 8, 12, and 16 days after radiolabel exposure. (3) Clearance of particles with a long-lived radiolabel (^{134}Cs) was measured after 24 months of diesel exposure to evaluate late phase (half-times in months to years) clearance. Radioactivity levels from ^{134}Cs were measured at 0.5 hr, and 3, 6, 10, 13, 18, 26, 110, 137, 164, and 194 days after radiolabel exposure.

Lung burdens of diesel exhaust particles at all levels showed a progressive increase with time. Lung burdens in the 3.5 and 7.0 mg/m^3 groups increased by 5- to 11-fold from 6 to 24 months of exhaust exposure while those of the 0.35 mg/m^3 group increased only 2.5-fold over the same period. The lung burdens were 0.6, 11.5 and 20.5 mg following 24 months of exposure at 0.35, 3.5 and 7.0 mg/m^3, respectively.

No significant differences were observed in the retention indicator of early clearance at any exposure time in any exposure group. There were exposure-related increases of intermediate clearance half-times in the 3.5 and 7 mg/m^3 exposure groups. Significantly longer intermediate clearance half-times were seen as early as 6 months in the 7.0 mg/m^3 group and at 18 months in the 3.5 mg/m^3 group. No significant changes were seen in the 0.35 mg/m^3 group. Significantly longer long-term clearance half-times were observed in the 3.5 and 7.0 mg/m^3 groups (240 - 264 days vs 79 days in controls). No significant changes were noted in the 0.35 mg/m^3 group (long-term clearance half-time of 81 days).

The pattern of clearance impairment was qualitatively in agreement with the observed lung burdens of diesel exhaust particles. According to the authors, for the 3.5 and 7.0 mg/m^3 groups, a reasonable approximation of the data could be made if pulmonary clearance remained normal for 9 months and then there was a rapid linear transition by 12 months to sequestering or nonclearance of particles (Wolff *et al*., 1987). Complete or near complete cessation of clearance was required to fit the observed data past 12 months. This sequestering response would mean that, after 12 months, pulmonary clearance half-times of diesel exhaust particles would approach infinity and certainly be much greater than the 240 to 260 day half-time observed for the ^{134}Cs tracer particles. Clearance half-times of nearly 500 days or more for pulmonary clearance of ^{14}C-diesel particles can be estimated for lung burdens of > 11.8 mg based on data from Chan *et al*. (1984). It is possible that the half-times for diesel exhaust particle clearance are underpredicted by ^{134}Cs particles. This could be the result of differences in particle size (2 μm vs 0.25 μm) and composition. It is also possible that the clearance observation time of 194 days was not long enough to predict rate of clearance of material which might have resided in the lung for as long as 2 years.

Wolff *et al*. (1986) reviewed the available data regarding lung retention of diesel particles and suggested that there may be thresholds of chronic diesel exposure in rats below which lung clearance and lung burden accumulations are normal or near normal. Above that threshold, lung clearance half-time generally increases with increasing lung burden. As indicated by the data above, impairments in clearance at high exposure concentrations are substantial and have suggested the incorporation of "sequestered" compartments in mathematical models of diesel particle clearance to account for the observed increases in lung burdens with long-term exposures. A sequestered compartment is consistent with the histopathological observations of numerous foci of particle-laden macrophages within areas of interstitial fibrosis.

Experimental studies in animals pre-exposed to carbon black particles suggest that the effect on lung clearance is primarily due to a particle effect (Lee *et al*., 1987; Strom, 1989). Exposure to high doses of carbon black increased lung retention of diesel-exhaust test particles in the same way that exposure to diesel exhaust at comparable particulate concentrations and duration did (Lee *et al*., 1987). Morrow (1988) used Fischer 344 rats to examine the possible mechanisms of particle overloading in the lung. Particle overloading was represented by a progressive reduction of particle clearance from the deep lung. Based on the study results, the author hypothesized that the breakdown in alveolar macrophage (AM)-mediated particle removal was due to the loss of AM mobility. The inability of the particle-laden AMs to translocate to the mucociliary escalator was correlated with an average composite particle volume per AM in the lung. When the particle volume exceeded approximately 60 μm^3/AM the overload effect was initiated. When the distributed particle volume exceeded approximately 600 μm^3/AM it appears that AM mediated particle clearance virtually ceases and aggregated particle-laden macrophages remain sequestered in the alveolar region. With chronic particle exposure, the increase in lung burden results in a progressive increase in the immobilized AM population, up to a burden at which virtually all AM-mediated clearance ceases. The heavily burdened macrophages may lyse in the alveoli and release the particles and cellular components which may injure type I cells and provoke proliferation of type II cells and further reduce the clearance by alveolar macrophages (White and Garg, 1981). Morrow (1988) emphasized that lung overloading per se does not depend upon the inherent chronic toxicity of the investigated particle. Whether the particle is tumorigenic, fibrogenic, or completely benign, dust overloading superimposes its actions of modifying both the dosimetry and the toxicological effects of the particles.

3.2.3. EXTRAPOLATION OF LUNG CLEARANCE ACROSS SPECIES

Although deposition efficiencies of airborne particles in the respiratory tract do not seem to vary much across species, the rate of clearance beyond the first day of exposure exhibits significant species differences. Based on the limited data available, the species clearance rates of small particles, such as those in diesel exhaust, from the lung appear to be (in order of fastest to slowest): hamster > mouse/rat > guinea pig/dog/human (Heinrich *et al*., 1986a; Lee *et al*., 1983; Pepelko, 1987; Oberdorster and Pott, 1986; Snipes *et al*., 1983). Differences in alveolar clearance rates are of prime importance for estimating the bioavailable dose from inhaled particles when extrapolating from one species to another. The potential magnitude of the dose

adjustment required when extrapolating to humans has been inferred by several of these investigators (Snipes *et al*., 1983; Oberdorster and Pott, 1986; Pepelko, 1987).

Oberdorster and Pott (1986) used model calculations to predict the deposition and retention of the small particles in environmental tobacco smoke (ETS, MMAD 0.2 μm) at an air concentration of 0.13 mg/m^3. Predictions of mass deposition rates per airway surface area were equal in rats and humans in the terminal bronchi but the rates for rats were up to 2-fold higher in other more proximal and more distal airways. Cadmium, a trace contaminant of ETS particles, was utilized to indicate accumulation. At equilibrium (when the amount deposited daily equals the amount being cleared from the lung) the amount accumulated per gram of lung was 4-fold greater in humans than in rats. The rate of approach to equilibrium was much slower in humans than in rats. Equilibrium was reached after approximately 10 years in humans vs 1 year in rats.

Pepelko (1987) examined the feasibility of dose adjustment based on differences in long-term clearance rates of inhaled particles in humans and laboratory animals. After evaluating the available literature, Pepelko suggested that when particles exhibit dissolution half-times of greater than 1 to 2 months (e.g. insoluble particles) a two to three-fold upward adjustment of retained lung burden dose should be considered when extrapolating from mice, rats and hamsters to humans for quantitative risk assessment. This adjustment factor is slightly lower than the approximate four-fold factor suggested by the results of Snipes *et al*. (1983) and Oberdorster and Pott (1986).

3.2.4. MATHEMATICAL MODELS FOR RETENTION

Stöber *et al*. (1989) proposed a model for the estimation of lung burden following long-term inhalation of particulates at high doses, leading to overload. In a physiology-oriented compartmental kinetic (POCK) model they took into account particle deposition rates, macrophage phagocytosis, and mucociliary clearance. The model contains 3 major compartments: lung, tracheobronchial tract, and lymphatic system. The lung compartment is divided into 3 smaller compartments: Alveolar surface, macrophage pool, and interstitium. One aspect of the model is its consideration of the central role of the macrophage in particle retention through phagocytosis and subsequent mobilization. As the lung burden increases above certain critical levels, the mobility of the active macrophage is retarded (Stöber *et al*., 1990b). Initial simulations using this model indicated a high degree of compliance with empirical data (Stöber *et al*., 1990a). Further comparisons with experimental bronchoalveolar lavage data allowed for refinement of the POCK model through replacement of an average macrophage load value with a macrophage load distribution (Stöber and Koch, 1991). More recently Stöber and Mauderly, (1994) also took into account lymph node saturation at high doses. The models were based on empirical data from various rat studies showing positive incidences of lung tumors in rats exposed to diesel exhaust. Although this model does not attempt to estimate lung burden at low concentrations, it is effective at modeling the kinetics of diesel particulate deposition and clearance at moderate and high diesel-particle concentrations.

A three-compartment model for diesel particle deposition and clearance has been developed by Yu and Yoon (1990) and includes parameters for estimation of inhaled particle transport from the lung into the blood, lymphatics and gastrointestinal tract. In addition, the Yu and Yoon model takes into account retardation of the mucociliary clearance mechanisms of the lung due to particle overload. The model mathematically describes the flow of the 3 particle components (carbon core, weakly-bound organics, and strongly-bound organics) among the compartments: lung, blood, lymphatics, and gastrointestinal tract.

3.3 PARTICLE ASSOCIATED ORGANIC COMPOUNDS

3.3.1 CLEARANCE OF PARTICLE ASSOCIATED ORGANICS FROM THE LUNG

Previous instillation studies found that tumor incidence from polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (BaP), increased when these compounds were associated with particles with prolonged retention (Henry and Kaufman, 1973; Sellakumar *et al*., 1976). Organic compounds generally account for 15-65% of the total particle mass in diesel exhaust (Cuddihy *et al*., 1984). Most organic compounds are relatively soluble in the lungs and can be cleared by direct absorption through the pulmonary epithelium into the blood (Sun *et al*., 1988). In contrast, deep lung clearance of relatively insoluble particles, such as diesel exhaust particles which carry adsorbed organics, depends primarily on the phagocytic activity of pulmonary macrophages.

At least four major classes of organic compounds are found associated with particles in exhaust (Sun *et al*., 1988):

1) aliphatic hydrocarbons and their oxidation products;
2) aromatic compounds, and their oxidation products;
3) alkyl-substituted aromatic compounds and their oxidation products; and
4) nitroaromatic compounds.

Among the organic compounds listed above, the most commonly studied ones are the nitro-PAHs, such as nitropyrene (NP) and the unsubstituted PAHs, such as BaP.

Factors influencing the bioavailability of adsorbed organics from diesel particles include: (1) the surface structure of the particle, (2) the composition of the adsorbed organic compounds, (3) the composition of the extracellular and intracellular fluids, (4) the balance of the molecular binding forces between the particle and the adsorbed organic molecules, and the particle and the extracting biologic fluids, and (5) the metabolism of the desorbed chemical (Gerde *et al*., 1991). The binding energies of the vapor to the particle probably determine the extent of bioavailability.

A series of experiments have been carried out at the Inhalation Toxicology Research Institute (ITRI) in Albuquerque, NM to evaluate the fate of inhaled 1-NP and BaP when adsorbed on gallium oxide or diesel exhaust particles as compared to the inhaled pure compound following acute exposure (Sun *et al*., 1982, 1983, 1984, 1988). Particles carrying adsorbed organic compounds deposit in the respiratory tract of rats by the same mechanisms as particles without adsorbed compounds. Inhaled aerosols of BaP or 1-NP alone, BaP or 1-NP adsorbed onto diesel

exhaust or gallium oxide particles deposit along the respiratory tract in about the same pattern as uncoated diesel exhaust or gallium oxide particles alone (Sun *et al*., 1988). Mucociliary clearance of particles in the ciliated airways and absorption into the blood of lipid soluble organics can rapidly clear material from the lungs; so it can be difficult to distinguish between the two mechanisms experimentally. However, studies in rats treated with radiolabeled pure organic aerosols or with the same radiolabeled organic compound adsorbed onto inorganic particles have shown that little radioactivity can be detected in the stomach following exposure to pure organic aerosols, but that substantial amounts of particle-associated radiolabeled organic material are detected in the stomach (Sun *et al*., 1982, 1983, 1984, 1988). It appears that the pure organic compounds deposited in the lung clear primarily by absorption through the respiratory epithelium into the blood. Particle-associated organic compounds clear by different routes (e.g. gastrointestinal tract), thereby exposing different tissues of the body.

Particle-associated organic compounds clear the upper respiratory tract (short-term phase) approximately as fast as pure organic aerosols (Sun *et al*., 1982, 1983, 1988). However, the late phase clearance rates of particle-associated organic compounds are longer than for inhaled pure organic compounds. The cause is presumed to be the tenacity with which the organic material is bound to the more slowly cleared "carrier" particles (Sun *et al*., 1988).

The lung retention/clearance curves from the available studies were summarized by Wolff *et al*. (1986) and Sun *et al*. (1988). One should note that these curves are based on acute exposure studies. Repeated, long-term exposure studies have not been conducted.

In the alveolar region the long-term retention of ^{3}H-BaP adsorbed on diesel particles was approximately 230-fold greater than that of the pure ^{3}H-BaP aerosol (Sun *et al*., 1984). Exposure of rats to ^{3}H-BaP associated with gallium oxide (Ga_2O_3) particles showed a 3 to 4-fold increase in the lung retention of ^{3}H-BaP compared to pure ^{3}H-BaP aerosol (Sun *et al*., 1982). Lung retention of ^{3}H-BaP/diesel exhaust particle was substantially greater than the inhaled ^{3}H-BaP/gallium oxide particle. This suggests that the composition of the carrier particle has an effect on the rate of lung clearance of ^{3}H-BaP. The lung retention of gallium oxide and diesel exhaust particles are relatively similar (see above subsection and Table 3.1 and 3.2). The rate limiting step of lung clearance of inhaled particle-associated ^{3}H-BaP may be the rate at which ^{3}H-BaP is removed from the carrier particle prior to lung clearance of the particle (Sun *et al*., 1984). It must be pointed out that different air concentrations of BaP were utilized in the different studies. Whether this affected the lung clearance rate is not known.

Long-term retention of ^{14}C-1-NP adsorbed on diesel particles was approximately 80-fold greater than that of pure ^{14}C-1-NP aerosols (Bond *et al*., 1986c). As with ^{3}H-BaP, lung retention of ^{14}C-1-NP/diesel exhaust particle was greater than the inhaled ^{14}C-1-NP/gallium oxide particle retention, although the difference was not as large.

Data from Sun and McClellan (1984) further supports the findings that particle-associated organics are cleared more slowly from the lungs than are pure organic aerosols. Radiolabeled (^{14}C) diesel fuel was used to generate exhaust in which the majority of the ^{14}C was with the organic compounds associated with the carbonaceous core particles. The radiolabeled exhaust

particles were intratracheally instilled into rats and the lung clearance of ^{14}C was measured. For comparison an extract of the radiolabeled organics from the exhaust particles was also instilled into the lungs of rats. These particle-free organic compounds cleared much faster than the particle-associated organic compounds. The distribution and kinetics of the particle-associated organics in organs other than the lungs was largely unchanged. Some release of particle-associated 1-NP and BaP apparently did take place since the retention of these compounds, though prolonged, was less than that of the diesel particles themselves.

The available evidence suggests that the effect of adsorption on diesel particles is to prolong the retention in the lung. This retention appears to be governed, at least in part, by factors related to the binding of the organic material to the particles.

3.3.2 ABSORPTION FROM THE GASTROINTESTINAL TRACT FOLLOWING PARTICLE TRANSLOCATION

During the rapid phase of clearance particles are translocated from the lungs to the gastrointestinal tract (see Section 3.2). Particle-associated organic compounds could potentially be absorbed into the body from the gastrointestinal tract following inhalation exposure.

To date, only one study has examined the gastrointestinal absorption of organics, specifically nitropyrene (NP), bound to diesel exhaust particles (Bond *et al*., 1986c). Rats were administered ^{14}C-NP in three different modes: 1) ^{14}C-NP suspended in saline administered orally (10 μg/kg); 2) ^{14}C-NP suspended in saline administered by intravenous (i.v.) injection (10 μg/kg); and 3) ^{14}C-NP coated on diesel exhaust particles administered orally (10 μg NP/kg). Urine and fecal excretion were monitored to determine the amount of absorption.

Gastrointestinal absorption of ^{14}C-NP and ^{14}C-NP coated on diesel exhaust particles was approximately 90%, suggesting that ^{14}C-NP deposited in the upper respiratory tract and cleared to the gastrointestinal tract will largely be absorbed into the body. A fairly large percentage (40%) of the absorbed ^{14}C-NP was metabolized in the liver, eliminated in the bile and was not reabsorbed but was excreted in the feces (Bond *et al*., 1986c).

3.3.3 METABOLISM OF PARTICLE-ASSOCIATED ORGANICS

Sun *et al*. (1988) has reviewed the metabolism of particle-associated compounds in automotive exhaust. The following section is, in large part, based on this comprehensive review. Many investigators believe that diesel particulate matter does not require metabolic activation for mutagenic activity. However, other studies have shown mutagenic effects with and without activation. See Section 5 for additional information.

Mixed-function oxygenases (MFO) are responsible for some of the metabolic conversion of procarcinogens including some PAHs. The MFO system is NADPH-dependent and catalyzes the incorporation of molecular oxygen into substrate molecules. Some relevant classes of procarcinogens which are adsorbed on airborne particles include PAHs, nitro-PAHs, aromatic

amines, nitrosamines, and N- or S-containing heterocycles. PAHs can be enzymatically converted to epoxide intermediates, which can spontaneously rearrange to phenols and then be converted enzymatically to trans-dihydrodiols via epoxide hydrolase. The trans-dihydrodiols may be reduced back to the parent compound via epoxide reductase, be conjugated nonenzymatically with glutathione or enzymatically conjugated via glutathione S-epoxide transferase, or react directly with cellular macromolecules. Alternatively, trans-dihydrodiols may be further oxidized to the diol epoxides which also react with cellular macromolecules. Sun *et al*. (1988) describe the major known metabolic pathways of BaP, a well studied PAH.

The metabolic activation of nitro-PAHs has been shown to involve nitroreduction, nitroaromatic ring-oxidation, N-hydroxyarylamine O-acetylation, or in some instances, a combination of all three pathways (Wislocki *et al*. 1986; Williams and Lewtas, 1985; Lewtas and Williams, 1986). Studies with nitroreductase-deficient strains of Salmonella typhimurium showed a substantial reduction in the mutagenicity, suggesting that nitrated compounds account for a significant portion of the mutagenic activity in the Ames test (Lewtas and Williams, 1986). Deconjugation and nitroreduction of nitro-PAHs has been shown to occur within the microflora of the lower intestinal tract (Ohnishi *et al*., 1986; Moller *et al*., 1987, 1989; Ball and King, 1985a,b; Ayres *et al*., 1985). Because of its nitro-reduction and conjugate-hydrolyzing activity, the gut microflora makes the first-pass biliary metabolites available for enterohepatic circulation (Ball and King, 1985a,b). Studies utilizing normal and antibiotic-treated rats found that abolition of gut microflora metabolism by antibiotic treatment significantly decreased the amount of macromolecular covalent binding of nitropyrene and its metabolites in tissue (Ayres *et al*., 1985). The metabolic activity of the gut flora is of special interest in light of the fact that a large percentage of inhaled diesel exhaust particulate is translocated to the intestinal tract. Sun *et al*. (1988) also presented some of the metabolic pathways for nitropyrene (NP), a typical nitro-PAH.

Aromatic amines, like nitro-PAHs, can be metabolized to N-hydroxyl intermediates. Nitrosamines are metabolized to unstable hydroxyl (gamma) carbon compounds that decompose to highly electrophilic N-monoalkylnitrosamines. Sun *et al*. (1988) present some of the known metabolic pathways for aminopyrene, a typical aromatic amine.

Biological activation of procarcinogens can occur in a variety of tissues. With few exceptions, the quantity of metabolites formed by lung tissue is less than that by the liver. However, since the respiratory tract is directly exposed to particle-associated carcinogens, metabolic activation by respiratory tract tissues may have an important role in the pathogenesis of carcinogen-induced lesions in these tissues. In vitro and in vivo studies indicate that nasal tissue can metabolize some particle-associated organic compounds to phenols, quinones, dihydrodiols and tetrols (Sun *et al*., 1988). Bronchial metabolism studies suggest that bronchial tissue in vitro is capable of metabolizing BaP to several compounds (e.g. phenols, quinones, and dihydrodiols) which can bind to DNA (See original references cited in Sun *et al*., 1988). Studies on tracheal metabolism are not as extensive; however they also suggest that tracheal tissue in culture is capable of metabolizing BaP to oxidative and conjugated metabolites which covalently bind to tracheal DNA.

Based on studies utilizing lung homogenates, perfused lungs, lung slices, and cultured type II alveolar cells, BaP and other PAHs are readily metabolized by lung tissue (Sun *et al*., 1988). The lung tissue systems listed above produced a variety of metabolites, including intermediates capable of covalently binding to DNA. In addition to the in vitro lung tissue systems, several studies have confirmed metabolism of BaP and NP in vivo following inhalation exposure (Bond *et al*., 1986b; 1986c; Sun *et al*., 1984). Metabolism of BaP and NP associated with diesel exhaust particles or in pure form was evaluated. The results demonstrated that lung tissue is capable of metabolizing (i.e. oxidizing, reducing and conjugating) BaP and NP in pure form or as constituents of diesel exhaust particles. In the case of BaP coated on diesel exhaust particles, metabolites were also detected in the lungs up to 20 days after a 1 hour exposure, although the total quantity of metabolites was considerably reduced compared to NP. The observation that lungs can metabolize BaP and NP to oxidized products suggests that during the formation of these metabolites, reactive intermediates are formed. Reactive intermediates could potentially bind to DNA, RNA and protein of lung tissue.

To study the factors affecting bioavailability of particle-associated PAHs, Leung *et al*. (1988) studied the ability of lung microsomes to facilitate transfer of BaP adsorbed on the surface of diesel exhaust particles to the microsomes, and the ability of the microsomes to metabolize the transferred BaP. The results from this study indicated that rat lung and liver microsomes were able to facilitate the transfer of a small fraction (< 3%) of the BaP from diesel exhaust particles, and that only a small percentage of the amount transferred was metabolized. Lung microsomes were about twice as effective as liver microsomes for the transfer of BaP. The ability to transfer BaP to the microsomes was independent of metabolism or the presence of protein, but was related to the lipid content of the microsomal fraction. There did not appear to be any metabolism of the BaP which remained coated on the diesel exhaust particles. Some particles deposited in lungs are phagocytized by macrophages. Some of the macrophages with engulfed particles remain in the lung for extended periods of time and slow release of organic compounds and their metabolites from these macrophages would subject surrounding tissue to extended exposure to potentially toxic or carcinogenic metabolites.

The metabolism of PAHs has been studied in the pulmonary macrophages of humans and of laboratory animals (Sun *et al*., 1988; Bond *et al*., 1984). BaP was commonly utilized as a model compound in these studies. Although the amount metabolized per unit of incubation time (i.e. metabolic rate) was low, the results indicated that macrophages can activate BaP and dimethylbenz[a]anthracene (DMBA) to reactive intermediates. The macrophages also released these metabolites into the surrounding medium which in vivo would result in exposure to the surrounding respiratory tract tissue. The metabolites formed from the pulmonary macrophage metabolism of particle associated BaP were not different from those observed for metabolism of the pure compound alone (Bond *et al*., 1984).

3.3.3.1 EFFECT OF PARTICLE PRE-EXPOSURE ON LUNG METABOLISM OF ORGANICS

Warshawsky and coworkers (1978, 1983, 1984 as cited by Sun *et al*., 1988) showed that the presence of particulate matter in the perfused lung can enhance the metabolic activation of BaP.

In particular they found that the levels of dihydrodiols were higher in the lungs from animals pre-exposed in vivo to particles than in animals that had not received particle preloading. Bond *et al*. (1985) examined the effect of pre-exposure to diesel exhaust particles on 1-NP metabolism in the whole animal. Rats were exposed to 0.35, 3.3 or 7.4 mg/m^3 of the particles for 7 hour/day, 5 day/week, for 4 weeks. After exposure, nasal and lung tissues were assessed for ^{14}C-1-NP metabolizing ability. Exposure to 7.4 mg/m^3 resulted in a significant increase (approximately twofold) in rates of NP metabolism in both nasal and lung tissues. Lower exposure concentrations did not significantly affect NP metabolism rates. A fourfold increase in the amount of ^{14}C covalently bound in the lung was also observed in the 7.4 mg/m^3 group.

Cantrell *et al*. (1981) also examined the effect of diesel exhaust exposure on the in vivo metabolism of BaP. Mice exposed to diesel exhaust and unexposed mice were intratracheally instilled with ^{3}H-BaP. Tissue radioactivity was determined 2, 24 and 168 hours (i.e. 7 days) later. The mice exposed to diesel exhaust appeared to have less free BaP in tissues, suggesting an induction of the enzymes for primary metabolism of polycyclic aromatic hydrocarbons. The group exposed to diesel exhaust and the unexposed group were both capable of clearing the bound radioactivity. However, there was a significantly higher amount of residual BaP in the lungs of the group exposed to diesel exhaust compared to the group of nonexposed animals at the 168 hour determination. The authors suggested that the higher proportion of BaP in diesel-exhaust exposed animals was due to the adsorption of BaP on particles.

Wolff *et al*. (1986) investigated the effect of pretreatment with diesel exhaust particulate extracts on the lung metabolism of 1-NP, BaP, and a mixture of DNP and 1,3,6-tri-NP in mice. The covalent binding to lung DNA was increased for all compounds by pretreatment, with the largest increase being greater than 2-fold for BaP.

3.3.4 DISTRIBUTION AND ELIMINATION OF PARTICLE-ASSOCIATED ORGANICS

The distribution and elimination of diesel particle-associated organics, specifically BaP and NP, have been investigated in several studies (Sun *et al*., 1982, 1983, 1984; Bond *et al*., 1986c; Lee *et al*., 1983). Clearance rates of radiolabeled pure compounds compared to compounds adsorbed onto particles suggested that a substantial amount of the particulate-associated organics cleared from the lungs by mucociliary clearance entered the blood from the gastrointestinal tract, whereas the majority of each of the pure organics cleared by direct absorption into the blood. The ultimate fate of the majority of each of the radiolabeled organics and their metabolites was excretion in feces. However, it appeared that the clearance of particle-associated organics by ingestion resulted in an increased dose to the stomach, liver, and kidneys when compared to exposure to the pure compound.

The organ which exhibited the greatest difference in radiolabel concentration was the lung (Bond *et al*., 1986c). Within 1 hour after exposure the lung from animals exposed to diesel particulate-associated ^{14}C-NP contained nearly 5 times more radiolabel than lungs from rats exposed to pure ^{14}C-NP aerosol. This difference was increased to 80-fold by 94 hours after exposure.

A variety of methods have been utilized to evaluate the elimination of diesel exhaust particles (Chan *et al*., 1981; Bond *et al*., 1986b; 1986c; Sun *et al*., 1983, 1984). Approximately 35 to 40% of the initial lung deposition was excreted in the feces during the first few days after exposure. This observation demonstrates the removal of diesel exhaust particles by mucociliary clearance in the upper airways and subsequent passage through the gastrointestinal tract. Fecal excretion was the major route of elimination, with approximately 2 times more excreted by this route than by the urinary route. Preliminary work with ^{14}C-NP bound to exhaust particles by Bond *et al*. (1986b; 1986c) has indicated that no ^{14}C was exhaled as $^{14}CO_2$ or unmetabolized ^{14}C-NP.

3.4 BIOMARKERS ASSOCIATED WITH DIESEL EXHAUST EXPOSURE

Kanoh *et al*. (1993) investigated the potential use of urinary 1-hydroxypyrene (1-HP), a metabolite of pyrene, as a biomarker for exposure to PAHs in diesel exhaust. Rats were exposed to diesel exhaust containing particulate matter at a concentration of 4.2 mg/m^3 for 8 weeks. 1-HP levels in 24-hour urine collections were then determined at 2, 4 and 8 weeks post-exposure. Urinary 1-HP levels were significantly increased in the diesel exhaust-exposed group compared to controls (2.4- and 5.6-fold at 2 and 4 weeks postexposure, respectively).

The use of an immunoassay procedure for determining urinary PAH and nitro-PAH content as a biomarker for exposure to diesel exhaust was developed by Scheepers *et al*. (1994). Total suspended particulate matter was sampled in 2 locations in a diesel locomotive repair shop. The 1-nitropyrene (1-NP) content was determined to vary from nondetectable to 5.6 ng/m^3. Urine samples from 3 diesel mechanics and 2 office clerks were then analyzed for 1-aminopyrene (1-AP; a rodent metabolite of 1-NP) using an 1-AP antibody binding assay. The cumulative and average excretion of 1-AP was significantly increased in the diesel mechanics when compared to the office clerk controls. These data strongly suggest that 1-AP may be useful as a biomarker of diesel exhaust exposure, and that nitroPAHs contained in diesel exhaust particulate matter may be bioavailable in humans.

3.5 SUMMARY OF TOXICOKINETICS

The emissions from diesel engines consist of both gaseous and particulate fractions. Particles in diesel exhaust have carbon cores upon which as many as 18,000 different substances, including organic compounds such as PAHs, are adsorbed. The disposition of the inhaled particles and their associated compounds is influenced by the processes of deposition, metabolism, distribution and clearance in the body, particularly the lung.

The deposition of diesel particles or surrogate diesel particles has been studied in rats, guinea pigs and dogs utilizing radiolabeled particles. Deposition of diesel exhaust in humans has been mathematically modeled. The pulmonary deposition efficiency appears not to vary much across the species studied thus far. In general, deposition efficiencies fall in the range of 12 to 20%.

Various respiratory tract tissues have been shown to metabolize the particle associated compounds, benzo(a)pyrene (BaP) and nitropyrene (NP). Further studies have demonstrated that the presence of or pre-exposure to particulate matter can enhance the metabolism of BaP and NP. Clearance of deposited particles is conveniently characterized in three phases. The short-term phase represents a rapid removal of particles deposited in the tracheobronchial tree or in the proximal respiratory bronchioles via the mucociliary escalator. The intermediate and late phase involve the removal of particulate matter from the alveoli following engulfment by macrophages. The intermediate phase occurs for normal removal, while the late phase occurs during impaired removal.

Early clearance appears to be similar across the species studied and independent of dose or exposure duration. Early clearance half-times generally range from 3 to 6 hours.

Intermediate-phase clearance rates appear to vary depending on species, dose and duration of exposure. Based on the limited data available on different species, the clearance rates of small particles, such as diesel exhaust, from the lung appear to be (in order of fastest to slowest): hamster > mouse/rat > guinea pig/dog/human. Lung clearance rates for mouse/rat are approximately 4 times greater than those for humans/dogs.

Under acute exposure protocols which do not disrupt normal alveolar clearance, the clearance half-times range from 40 to 80 days in the species studied to date (i.e. the rat). Repeated exposure to diesel particles for more than one month results in increases in pulmonary clearance half-times. Repeated exposure to sufficiently high concentrations results in excessive particulate loading. Particle-laden macrophages exhibit decreased mobility and phagocytic activity resulting in sluggish transport of particles from the lungs.

Table 3-1. Lung Deposition of Diesel Exhaust or Related Particles.

Exposure	Species	Observations	References
Nose-only exposure for 40-45 min. to radiolabeled diesel particulate (6 mg/m^3). Used radioactive tracers ^{131}Ba and ^{14}C. 14C-diesel particulate generated from a Farymann single cylinder diesel engine using 2D diesel fuel. ^{131}Ba-diesel particulate generated by GM 5.7 liter engine using 2D fuel. (MMAD 0.1 - 0.15 μm)	Fischer 344 male rats	Deposition in Lungs: ^{131}Ba tracer - 15% (10% alveolar, 5% tracheobronchial) ^{14}C tracer - 17% (11% alveolar, 6% tracheobronchial)	Chan *et al* J Appl Toxicol 1(2)77-82, 1981
Nose-only exposure to ^{14}C radiotagged diesel particles (MMAD 0.12 μm). Exhaust generated by a Farymann single cylinder diesel engine burning 2D fuel. Exposed to 7 mg/m^3 for 45 min. or to 2 mg/m^3 for 140 min.	Fischer 344 male rats	Lung Deposition (tracheobronchial and alveolar): 2 mg/m^3 for 140 min. - 20% 7 mg/m^3 for 45 min. - 17%	Lee *et al* J Toxicol Environ Health 12:801-813, 1983
Nose-only exposure to $^{67}Ga_2O_3$ (MMAD 0.1 μm) for 30 min. Air concentration not reported. Radioactivity measured at 0.5 hr, and 1, 2, 4, 6, and 14 days after exposure.	Fischer 344 (SPF) rats	Lung Deposition: Nasopharyngeal - 9% Bronchial - 5% Pulmonary - 11%	Wolff *et al* Ind Hyg Assoc J 45(6)377-381, 1984
Deposition of $^{67}Ga_2O_3$ (0.1 μm MMAD) and ^{134}Cs-fused aluminosilicate particles (MMAD 2.0 μm) was measured following exposure to diesel exhaust (0, 0.35, 3.5 or 7 mg/m^3, 7 h/d, 5 d/wk for periods up to 24 months). Diesel particulates were generated by Oldsmobile V-8, 5.7 liter engine.	Fischer 344 rats	Pulmonary deposition values were similar for all exposure concentrations and at all time points. Average deposition: $^{67}Ga_2O_3$: / ^{134}Cs-FAP: 0 mg/m^3: 9.2% / 0 mg/m^3: 6.6% 0.35 mg/m^3: 12.6% / 0.35 mg/m^3: 7.7% 3.5 mg/m^3: 11.6% / 3.5 mg/m^3: 5.4% 7 mg/m^3: 10.7% / 7 mg/m^3: 8.1% Authors stated that the lower deposition rate for ^{134}Cs-FAP was probably due to larger particle size.	Wolff *et al* Fund Appl Tox 9:154-166,1987
Exposed to 6 mg/m^3 diesel exhaust or 7 mg/m^3 carbon black, 20 hr/d, 7 d/wk for 1 to 12 weeks. (MMAD for exhaust 0.19 μm; carbon black 0.24 μm)	Fischer 344 (male)	Based on compartmental modeling alveolar deposition was estimated to be 27% for diesel exhaust particles and 15% for carbon black particles.	Strom *et al* J Tox Env Hlth 26:183-202, 1989

Table 3-1. Lung Deposition of Diesel Exhaust or Related Particles (continued).

Exposure	Species	Observations	References
Nose-only exposure to ^{14}C radiotagged diesel particles (MMAD 0.12 μm). Exhaust generated by a Farymann single cylinder diesel engine burning 2D fuel. Exposed to 7 mg/m^3 for 45 min.	Hartley male guinea pigs	Lung Deposition - 20%	Lee *et al* J Toxicol Env Hlth 12:801-813, 1983
Nose-only exposure to $^{67}Ga_2O_3$ aerosols (0.1 μm size range)	Beagle dogs	Total deposition: 33% Nasopharyngeal region: 6% Tracheobronchial region: 6% Alveolar region: 21%	Brooks *et al* Env Int'l 5:263-267, 1981 and Wolff *et al* J Aerosol Sci 12:119-129, 1981
Nose-only exposure to $^{67}Ga_2O_3$ (MMAD 0.1 μm) for 30 min. Air concentration not reported.	Beagle dogs	Total deposition: 39% Nasopharyngeal - 7% Bronchial - 7% Pulmonary - 25%	Wolff *et al* Ind Hyg Assoc J 45(6)377-381, 1984
MMAD approx. 0.2 μm, geometric standard deviation 4.5	Mathematical lung model (human)	Calculated an alveolar deposition of 10 to 13%, under normal breathing conditions	Yu *et al* Health Effects Institute Research Report No. 10;3-27, 1987

Table 3-2. Lung Clearance of Diesel Exhaust Particles Following Acute Exposure.

Exposure	Species	Observations	References
Nose-only exposure to 6 mg/m^3 for 40-45 min to radiotagged diesel exhaust particulate. Utilized radiotracers ^{131}Ba and ^{14}C (MMAD 0.1 - 0.15 μm). Retention in the lungs was measured for up to 105 days postexposure.	Fischer male rats	Two distinct clearance phases were observed: Phase 1 half-time - 1 day (95% CI 0 - 2 days) Phase 2 half-time - 62 days (95% CI 35 - 89 days). 27% of the initial lung burden remained in the lungs at 105 days postexposure.	Chan *et al* J Appl Tox 1:77-82, 1981
Nose-only exposure to radioactive ^{14}C-diesel particles (MMAD 0.12 μm), 7 mg/m^3 for 45 min. or 2 mg/m^3 for 140 min. Lung clearance was determined at various time intervals up to 1 year postexposure.	Fischer 344 male rats	No significant difference in lung clearance was observed between the two exposure groups. Three clearance phases were seen: Phase 1 half-time - 1 day (tracheobronchial clearance) Phase 2 half-time - 6 days (proximal respiratory bronchiole clearance) Phase 3 half-time - 80 days (alveolar clearance)	Lee *et al* J Toxicol Env Hlth 12:801-813, 1983
Nose-only exposure to radioactive ^{14}C-diesel particles (MMAD 0.12 μm), 7 mg/m^3 for 45 min. Lung clearance was determined at various time intervals up to 1 year postexposure.	Hartley male guinea pigs	Very little clearance was observed from day 10 to day 432 postexposure. Only a half-time for the rapid clearance phase could be estimated (1 - 2 days).	Lee *et al* J Toxicol Env Hlth 12:801-813, 1983.

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation.

Exposure	Species	Observations	References
Exposed for 7 h/d, 5 d/wk for 18 weeks to 0.15, 0.94 or 4.1 mg/m^3 diesel exhaust particles. Lung burden was determined at 1 day and 1, 5, 33, and 52 weeks after termination of exposure.	Fischer 344 rats	Long term clearance half-times: 0.15 mg/m^3 - 87 days 0.94 mg/m^3 - 99 days 4.1 mg/m^3 - 165 days ($p<0.0001$) No control group or baseline clearance values were reported.	Griffis *et al* Fund Appl Tox 3:99-103, 1983
Following exposure to 0, 0.25 or 6 mg/m^3 diesel exhaust particles for 20 h/d, 7 d/wk for up to 112 days, animals were exposed, nose-only, to ^{14}C-tagged diesel particles for 45 min. (MMAD 0.17 μm). Radioactivity was then monitored for 1 year.	Fischer 344 male rats	Lung burden and overall alveolar clearance half-times (i.e. includes sequestered fraction): 0 mg/m^3 - 0 and 77 days 0.25 mg/m^3 for 52 days - 0.2 mg and 90 days 0.25 mg/m^3 for 112 days - 0.5 mg and 92 days 6 mg/m^3 for 7 days - 0.7 mg and 166 days ($p<0.05$) 6 mg/m^3 for 62 days - 6.5 mg and 562 days ($p<0.05$) 6 mg/m^3 for 112 days - 11.8 mg and no apparent clearance <u>Alternative approach</u>: Separate the alveolar clearance into two components: 1) active alveolar clearance; and 2) sequestered or residual fraction. Active alveolar clearance half-times were not significantly different across groups (45 to 71 days), except for 6 mg/m^3 for 62 days (148 days, $p<0.05$) and 6 mg/m^3 for 112 days (clearance rate not measurable). The residual component was estimated to represent 7, 13.3, 18, 45.5 and 88% of the initial ^{14}C particle deposition for animals exposed to 0.25 mg/m^3 for 52 days, 0.25 mg/m^3 for 112 days, 6 mg/m^3 for 7 days, 6 mg/m^3 for 62 days and 6 mg/m^3 for 112 days, respectively.	Chan *et al* Fund Appl Toxicol 4:624-631, 1984

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation (continued).

Exposure	Species	Observations	References
Following exposure to carbon black particles (MMAD 0.22 μm), 6 mg/m^3 for 20 h/d, 7 d/wk for up to 11 wks, animals were nose-only exposed to ^{14}C-tagged diesel particles for 45 min. Lung retention was monitored for up to 1 year. Diesel particles were generated by a GM 5.7 liter engine (MMAD 0.17 μm). (continued from previous page)	Fischer 344 rats	Prolonged exposure to carbon black particles resulted in an inhibitory effect on pulmonary clearance. Some particle-laden macrophages were observed sequestered as aggregates in the pulmonary region, the lung retention data were analyzed using a normal two-phase model with a sequestered term. Assuming that pulmonary retention at 1 year postexposure represented the amount sequestered, the long-term clearance half-times were noticeably higher in animals exposed for as little as 3 weeks to carbon black. Animals exposed for only 1 week exhibited half-times similar to controls. 1 week exposure: 57 days clearance half-time 3 week exposure: 96 days clearance half-time 5 week exposure: 140 days clearance half-time Half-time for exposure > 5 weeks were even longer, but actual values were not reported.	Lee *et al* Env Res 43:364-373, 1987

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation (continued).

Exposure	Species	Observations	References
After 2 months of exposure to 0 or 2 mg/m^3 diesel exhaust (7 h/d 5 d/wk) animals were exposed nose-only to $^{59}Fe_3O_4$ for 2 hours (15.4 mg/m^3, MMAD 1.5 μm)	Fischer 344 weanling male rats	Beyond day 1 the lung clearance could be described by a monoexponential curve with a half-time of 37 days in diesel exposed animals and 47 days in controls. Diesel exposed animals had a significantly more rapid clearance rate than controls. 8% of the initially deposited $^{59}Fe_3O_4$ particles remained in the lungs at 120 days postexposure in diesel exposed animals vs. 19% in controls.	Lewis *et al* In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Ed: N Ishinishi *et al* pp. 361-380, 1986
Following exposure to 4 mg/m^3 diesel exhaust particles 19 h/d, 5 d/wk for 3 to 19 months animals were exposed to $^{59}Fe_2O_3$ aerosol. Radioactivity was monitored for 80 days.	Wistar (SPF) female rats	Average control clearance half-time was 49 days (range 40 - 68 days). Exposed animals exhibited a significantly longer half-time after only 3 months of diesel exposure. The half-time was 2.5-fold greater than controls. Continued exposure to diesel did not further increase the clearance half-time. The values after 12 and 19 months were slightly lower than after 3 and 8 months.	Heinrich *et al* J Appl Toxicol 6:383-395, 1986
Following exposure to 3.9 mg/m^3 diesel exhaust particles, 7-8 h/d, 5 d/wk for 18 months, animals were exposed to $^{59}Fe_2O_3$ radioaerosol.	Wistar (SPF) female rats	Average control clearance half-time was 48.5 $\pm$ 4.2 days. Average exhaust-exposed half-time was 92.4 $\pm$ 14.3 days.	Heinrich *et al* J Appl Toxicol 6:383-395, 1986

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation (continued).

Exposure	Species	Observations	References
Animals were exposed for 7 h/d, 5 d/wk for up to 30 months to 0, 0.35, 3.5 or 7 mg/m^3 diesel exhaust particles. Lung burden and clearance were monitored at various time points up to 24 months. Radiotracers ^{99m}Tc, ^{67}Ga and ^{134}Cs were used to estimate clearance times. ^{99m}Tc-macroaggregated albumin was instilled onto the epithelium of the lower trachea at 3 wks prior to and at 6, 12, 18, 24, and 30 months of exposure. The percent of material retained at the original instillation site at 60 min. was calculated to provide an indicator of tracheal clearance.	Fischer (SPF) rats	Average lung burdens: 0.35 mg/m^3: 3 months - 0.13 mg 6 months - 0.23 mg 12 months - 0.24 mg 18 months - 0.32 mg 24 months - 0.6 mg 3.5 mg/m^3: 3 months - 1.2 mg 6 months - 1.04 mg 12 months - 2.18 mg 18 months - 7.78 mg 24 months - 11.49 mg 7.0 mg/m^3: 3 months - 2.70 mg 6 months - 4.1 mg 12 months - 7.29 mg 18 months - 15.76 mg 24 months - 20.47 mg	Wolff *et al* In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Ed: N Ishinishi *et al*. pp. 199-211, 1986 and Wolff *et al* Fund Appl Tox 9:154-166, 1987

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation (continued).

Exposure	Species	Observations	References
$^{67}Ga_2O_3$ (MMAD 0.1μm) - rats were exposed nose-only for 30 min. at various time points (6, 12, 18 and 24 mon) during diesel exposure. Radioactivity was measured at 30 min. and 1, 4, 8, 12, and 16 d after radiotracer exposure. ^{134}Cs-fused aluminosilicate particles (FAP) (MMAD 2.0 μm) - rats were exposed nose-only for 30 min. after 24 mon of diesel exposure. Radioactivity was measured at 0.5 hr and 3, 6, 10, 13, 18, 26, 110, 137, 164, and 194 d after radiotracer exposure.	Fischer (SPF) rats	^{99m}Tc-macroaggregated albumin retention values were significantly lower at pre-exposure times in all groups than during the exposure. There were no significant differences between control and any exposure group at any time point (Half-time clearance values were not reported). $^{67}Ga_2O_3$ clearance - observed 2 components: 1) Early clearance component half-times were not significantly different between controls and any exposure group. Half-times ranged from 0.5 to 1.4 days; and 2) Longer-term clearance component half-times exhibited an exposure related increase at the 3.5 and 7 mg/m^3 exposure levels. Significantly longer half-times were seen after 18 months at 3.5 mg/m^3 and after 6 months at 7 mg/m^3. Control half-times ranged from 36 to 47 days during the 24 month study period. 0.35 mg/m^3 half-times ranged from 35 to 46 days 3.5 mg/m^3 half-times gradually increased: 37, 60, 82* and 79* days at 6, 12, 18, and 24 months, respectively. (* $p<0.05$) 7 mg/m^3 half-times: 151*, 127**, 84 and 121* days at 6, 12, 18, and 24 months, respectively. (* $p<0.05$; ** $p<0.1$) ^{134}Cs-FAP clearance: observed two components. 1) Short-term component - no significant difference between controls or any exposure group. Half-times ranged from 1.5 to 1.8 days. 2) Longer-term component - 3.5 and 7 mg/m^3 exposure groups had significantly longer half-times than controls or 0.35 mg/m^3 exposure groups. Control: 79 days 0.35 mg/m^3: 81 days 3.5 mg/m^3: 264 days ($p<0.01$) 7 mg/m^3: 240 days ($p<0.01$)	ibid

Table 3-3. Lung Clearance Following Chronic and Subchronic Exposure to Diesel Exhaust by Inhalation (continued).

Exposure	Species	Observations	References
Starting at 13 months of age, animals were exposed to 0 or 6.34 mg/m^3 diesel exhaust particles (DEP) for 8 hr/d, 7 d/wk for 61 weeks, and then exposure was increased to 11.7 mg/m^3 DEP for the subsequent 62 weeks (a total exposure period of 27 months) Approximately half of the animals were killed at the end of exposure, the remaining half were placed in clean air for an additional 6 months and then terminated. Source of diesel exhaust was a Nissan CN6-33 engine burning number 2 diesel fuel.	cats	Diesel particle density within the interstitial macrophages of the cats was decreased by about 50% in the group which were maintained in clean air for 6 months following diesel exposure compared to those animals killed immediately after diesel exposure ended, suggesting a clearance half-time of roughly 6 months. This clearance half-time is longer than that of the rat but shorter than that of the guinea pig.	Hyde, *et al* Lab Invest 52(2):195-206, 1985
Following exposure to 4 mg/m^3 diesel exhaust particles 19 h/d, 5 d/wk for 12 months, animals were exposed to $^{59}Fe_2O_3$ aerosol. Radioactivity was monitored for 80 days.	Syrian golden hamsters	Exposed animals exhibited an insignificant decrease in lung clearance. Clean air controls exhibited a half-time of 55 days whereas exposed animals exhibited a clearance half-time of 75 days.	Heinrich *et al* J Appl Toxicol 6:383-395, 1986

4.0 NON-CANCER HEALTH EFFECTS

Together, this section and Appendix A review the literature of effects of diesel exhaust that are not specifically related to genotoxicity and carcinogenicity. Appendix A -- Reference Concentration for Chronic Inhalation Exposure (RfC): Diesel Engine Emissions; IRIS, U.S.EPA, 6/93 -- established the basis for the U.S.EPA reference concentration of 5 $\mu g/m^3$.
The first subsection below summarizes the available epidemiological literature on the potential health effects of diesel exposure. This evidence mostly derives from studies of diesel exhaust exposed workers. The next two subsections describe the results of animal testing. They focus primarily upon the potential for adverse respiratory and reproductive effects. The fourth subsection summarizes the immunological effects of diesel exhaust in both humans and animals. The fifth sub-section provides an evaluation of the U.S.EPA RfC and WHO's proposed health-based guidance values. This sub-section also includes OEHHA's calculations and recommends the adoption of the 1993 U.S.EPA RfC as the California chronic inhalation Reference Exposure Level (REL).

4.0.1 CHAPTER SUMMARY AND CONCLUSION

Epidemiological studies reviewed in this chapter provide evidence which suggests that diesel exposed workers had increased frequency of bronchitic symptoms, cough and phlegm, wheezing, and decrement in lung function. However, confounding factors present during exposure, often obscured the exposure-effect relationships in these studies.

In acute or subchronic animal studies, the inhalation or direct application of diesel particles into the respiratory system induced inflammatory airway changes, lung function changes, and increased susceptibility to lung infection. Chronic exposures caused changes such as thickening of the alveolar epithelium and infiltration of macrophages, fibroblasts and proteins into the alveolar septa, changes which are mainly related to chronic inflammatory responses.

There are no data available in the literature concerning reproductive and developmental health effects in humans. Diesel exhaust particles do not induced heritable point mutations and sperm abnormalities in rats, mice, and monkeys, though sperm anomalies were noted in exposed hamsters. Effect on the female reproductive system was limited to a study reporting a decrease in the number of corpora lutea in diesel exhaust particulate exposed female mice. Diesel exhaust is not teratogenic in rabbits.

In both humans and animals, diesel exhaust exposure can result in measurable increases in IgE and IgG antibody production, perturbed cytokine regulation, localized inflammation and eosinophilic infiltration in lung and respiratory tract tissues. In allergic human subjects, diesel exhaust particles may act as an adjuvant for pollen allergy.

In conclusion, there is evidence to suggest that human exposure to diesel exhaust causes chronic respiratory symptoms and contributes to the recent increase in allergic respiratory diseases. Results from animal studies provide support for this conclusion. The human health effects data

currently available do not allow for a quantitative derivation of a chronic inhalation REL. The OEHHA concurs with the U.S. EPA that the chronic rat study by Ishinishi *et al*. (1988) is the most appropriate study for this purpose and recommends 5 $\mu g/m^3$ as the chronic REL for diesel exhaust.

4.1 RESPIRATORY HEALTH EFFECTS

Epidemiological studies suggest increased frequency of bronchitic symptoms, cough and phlegm, wheezing, and decrements in lung function as measured by forced expiratory volume in workers exposed to diesel exhaust. Exposure-effect relationships in these studies are often obscured by confounding factors such as the presence of mine or coal dusts.

4.1.1 HUMAN STUDIES

The studies reviewed in this section involve primarily populations of miners exposed to diesel exhaust. The advantage of studying miners is that they are not concurrently exposed to exhaust from gasoline-powered vehicles, as are most transport workers. Because of the confined spaces in which miners work, they may be more highly exposed to diesel exhaust than any other occupational group. On the other hand, studies of miners raise the question of potential confounding by substantial concentrations of other substances, particularly dusts, to which they may have been concurrently exposed. Studies of road transport and road tunnel workers have not been included because of variable unknown exposures to gasoline exhaust. Measurements of gross respiratory effects in humans have not provided sufficient quantitative information to establish a reference concentration at the time of this review.

An early occupational study found no significant effects on health attributable to diesel exposed workers. Battigelli *et al*. (1964) obtained pulmonary function and frequency of respiratory complaints for 210 railway engine repairmen in comparison to 150 railway yard workers. This study also found little elevation of NO_2 and respirable particles in the general shop atmosphere.

The first study to examine the possible effects of diesel exposure on miners was a qualitative study by Jorgensen and Svensson (1970), which compared 60 Swedish underground iron ore miners in a mine where diesel powered vehicles were used extensively with 55 above-ground miners. No environmental measurements were made. As with many studies, the nature of the diesel fuel used here was likely somewhat different from modern diesel fuel; the report of this study in particular indicated that light-diesel and catalytic afterburners and/or waterscrubbers were used in the mine for several years. The frequency of bronchitic symptoms was higher among underground miners, even after adjustment for smoking status. Spirometry measurements revealed no differences between the two groups.

Ames *et al*. (1982) compared an exposed group of 60 U.S. coal miners from mines using diesel equipment with 90 miners not exposed to diesel emissions. The control subjects were on average 15.1 years older than the exposed group and had 15.9 more years experience underground. Decrements in FVC and FEV_1 were found in the exposed group compared to the controls, but

these decrements were not statistically significant. However, there is a suggestion of an important effect that might have been obscured because of the difficulty of detecting effects with the small number of subjects in this study and the greater age of the controls.

Attfield *et al*. (1982) studied 1091 miners in 6 New Mexico potash mines. They were unable to find any trends in respiratory signs and symptoms in relation to diesel use or NO_2 exposure. Again, important relationships could have been obscured because of differences in worker characteristics and concurrent exposures to other substances in mines.

In a cross-sectional study by Reger *et al*. (1982), 823 underground coal miners in mines using diesel-powered equipment were individually matched with miners working in coal mines above or below ground. These investigators found a significantly elevated incidence of cough and phlegm in underground diesel-exposed miners, but pulmonary-function test results (FEV_1 and FVC) were not different in a consistent trend from the matched control miners. Diesel-exposed workers had lower incidence of dyspnea compared with the control miners. The authors did not reach definitive conclusions about the influence of diesel exhaust on pulmonary function or pulmonary symptoms.

In another cross-sectional study, using 259 salt miners from 5 mines with varying diesel exposure, Gamble *et al*. (1983) reported that phlegm production was associated with exposure to respirable particles and NO_2 (assumed to be a surrogate for diesel exhaust), but cough, dyspnea and pulmonary function were not. Acute pulmonary function deficits were, however, observed after workshifts in workers who were smokers. In separate analyses of the same individuals, Gamble and Jones (1983a, 1983b) associated the increased phlegm production specifically with diesel exhaust. They (1983a) also reported a significant decrease of vital capacity with exposure to diesel exhaust, and their data suggest a trend of increasing dyspnea with diesel exhaust.

Robertson *et al*. (1984) studied 560 British coal miners, including 44 matched pairs with high and low NO_x exposure. Prevalence of respiratory symptoms and FEV_1 were investigated. No association was shown, either in the whole cohort or the matched group, between prevalence of respiratory symptoms or FEV_1 in relation to nitrogen oxides exposure. However, associations were shown for dust exposure and cigarette smoking.

In a five-year prospective study, Ames *et al*. (1984) tested the hypothesis that exposure to diesel exhaust leads to chronic respiratory effects among underground coal workers. Neither changes in respiratory function nor in chronic respiratory symptoms supported the hypothesis. The study employed three spirometric measures of respiratory function and three measures of chronic respiratory symptoms. In a further analysis, which included dust and gaseous exposure measurements for 337 of the exposed miners, Ames *et al*. (1988) came to similar conclusions for that subset of miners.

In a study of the pulmonary function of stevedores exposed to diesel exhaust, Ulfvarson *et al*. (1987) reported significant but reversible reductions in forced expiratory measures over the course of a working shift. Ten workers were tested on each of two occasions. Personal sampling gave dust levels from 0.13 to 1.0 mg/m^3, CO from 1.1 to 5 mg/m^3 and NO_2 from 0.06 to 2.3

mg/m^3. However, no correlations could be made regarding the concentrations of nitric oxide, NO_2, formaldehyde, or CO and pulmonary function deficits. The method of subject recruitment was not presented in the study. The number of individuals who experienced pulmonary function decline that were also smokers was not given, however the authors reported no group differences between smokers and non-smokers in this regard. Subsequently, Ulfvarson and Alexandersson (1990) studied the effect of removing the particles from the diesel truck exhaust by means of high-efficiency particle filters. The result was that forced vital capacity declined by 2% for the group of 24 when all trucks used filters and 5% for the group of 18 when none did. Tests of six subjects experiencing both kinds of days showed a decline of 5% for the filtered exposure compared to a decline of 10% for unfiltered exposure. Respirable dust declined from 0.23 without filters to 0.12 mg/m^3 with filters. In a similar study of 15 workers in a tunnel construction site, Ulfvarson *et al*. (1991) reported that filtering the diesel exhaust had a protective effect on FVC and FEV_1. Dalquist (1995) and Dalquist and Ulfvarson (1996) further reported that these tunnel workers in a workshift had "cross-shift" decreases in lung function which may be associated with eventual long-term decline in lung function. The authors concluded that the transient decrease in lung function seen in their studies was related to the diesel particulates and not to the gaseous components of DE. In diesel-bus garages Gamble *et al*. studied acute health effects of nitrogen dioxide and respirable particulate matter on 232 workers (1987a), and chronic respiratory effects of diesel exhaust on 283 workers (1987b). In the acute study they found measures of exposure to nitrogen dioxide and respirable particulate matter over a shift to be related to work-related symptoms of cough; itching, burning or watering eyes; difficult or labored breathing; chest tightness; and wheeze (Gamble *et al*., 1987a). In the chronic study, analysis of exposure-effect relationships within the study population showed no detectable associations of symptoms with tenure. There was an apparent association of pulmonary function and tenure. The study population was also compared to a nonexposed "blue collar" population. After indirect adjustment for age, race, and smoking the study population had elevated prevalence of cough, phlegm and wheezing compared to the nonexposed population, but there was no association with tenure. Dyspnea showed a dose-response trend but no increase in prevalence compared to the unexposed population (Gamble *et al*. 1987b).

In stevedores, Purdham *et al*. (1987) studied respiratory symptoms and pulmonary function in relation to components of diesel and gasoline exhaust exposure. The stevedore exposure to total suspended particulate matter and carbon monoxide was significantly greater than controls. Lung function assessments provided evidence that an obstructive ventilatory effect was more prevalent in the 17 stevedores than in the 11 controls.

Several of the studies of cancer risk also reported data on mortality from non-malignant respiratory disease. Howe *et al*. (1983) in railway workers and Wong *et al*. (1985) in heavy equipment workers both reported increases in mortality from emphysema. Cigarette smoking may have confounded those observations. In a preliminary report for railroad workers exposed to diesel exhaust, Garshick *et al*. (1987c) reported data giving evidence of a modest increase in mortality due to chronic respiratory disease, even after careful control for smoking.

The studies of Gamble *et al*. (1983), Gamble and Jones (1983a, 1983b), and Gamble *et al*. (1987a, 1987b), though not entirely consistent, suggest together that exposure to diesel exhaust

may be related to respiratory symptoms. The studies of Ulfvarson *et al*. (1987, 1991) and Ulfvarson and Alexandersson (1990) indicate occurrences of decrements in pulmonary function attributable to diesel exhaust over a workshift. Seven studies reported no relationship to pulmonary function or respiratory symptoms.

Rudell *et al*. (1996) performed acute, 1-hour exposures of 12 healthy, non-smoking non-asthmatic subjects to filtered and unfiltered diesel exhaust (1.4 - 2.6 x 10^6 particles/cm^3). The subjects underwent light exercise during the exposure, with periodic measurements of eye and nasal irritation. Pulmonary function was measured before and after exposure. Results included significant increases in total and specific airway resistance, and significant increases in symptoms of eye and nasal irritation, compared with controls exposed to exhaust-free air. The presence of a ceramic particle trap was effective in reducing the number of particles above 0.4 μm in diameter, but was ineffective in restoring impaired pulmonary function, or in abating symptoms.

Three cases of railroad workers with reactive airway disease were documented by Wade and Newman (1993). The workers, none of whom were current smokers, had no prior history of asthma or other respiratory disease. The workers developed asthma following excessive exposure to diesel emissions while riding behind the lead engines of caboose-less trains.

Recent studies indicate that diesel exhaust particles play a role in immunological allergic reactions as well as localized inflammatory responses in humans. This will be discussed below (Section 4.3).

4.1.2 SUMMARY OF HUMAN RESPIRATORY HEALTH EFFECTS

Epidemiological studies suggest increased frequency of bronchitic symptoms, cough and phlegm, wheezing, and decrements in lung function as measured by forced expiratory volume in workers exposed to diesel exhaust. The epidemiological studies summarized above all had clear limitations. They were commonly characterized by fairly small numbers of subjects, limited information on historical exposure levels, possible confounding from exposure to other substances, and potential for selection bias due to the possibility that the most affected workers had already left their employment before the beginning of the studies. The possibility of selection bias must also be considered since the method of recruitment of many subjects was not presented. Recall bias in questionnaire-type studies can also influence results in either direction. The degree to which these possible confounding variables influenced the results from the above studies is unknown. The studies in miners showed some limited association between diesel exhaust and respiratory symptoms but not pulmonary function tests. The studies of underground workers present particular difficulties in establishing an effect because of the potential confounding dust exposures and inherent difficulties in detecting increases in incidence rates relative to a high background rate

4.1.3 RESPIRATORY HEALTH EFFECTS IN ANIMAL STUDIES

Studies utilizing various routes of exposure are summarized in Tables 4.1, 4.2, 4.3, and 4.4. Only those studies utilizing inhalation exposure or a direct application of diesel into the respiratory tract, and evaluating the lowest levels producing effects will be discussed further in this section. Effects on reproduction and development will be discussed in a separate section (Section 4.2).

The inhalation or direct application of diesel into the respiratory tract of animals in acute and subchronic studies induced inflammatory airway changes, lung function changes, and increased susceptibility of exposed animals to lung infection. The morphological effects observed in the lungs of animals in chronic inhalation exposures are mainly related to chronic inflammatory responses.

4.1.3.1 ACUTE EXPOSURE (SINGLE DOSE)

The effects of acute exposure to diesel exhaust are summarized in Table 4.1.

Pepelko and Peirano (1983) evaluated the health effects of an acute inhalation exposure to diesel exhaust (DE). Mice were exposed for 2 or 6 hours to 6 mg/m^3 diluted exhaust from a light-duty diesel engine. No effects were observed after 2 hours of exposure, however, after the 6 hour exposure the infectivity of Streptococcus pyogenes was significantly enhanced. The average mortality in the 6-hour DE exposed animals was 31.7% compared to 6.7% in control animals. The highly positive results of this study show that brief exposure to diesel exhaust at the exposure level of a 6 mg/m^3 greatly enhances the infectivity of one of the bacterial challenges. The overloaded clearance mechanism at this concentration is a likely mechanism of interference with lung defenses.

A single acute intranasal exposure of guinea pigs to solutions of suspended diesel particles resulted in significant increases in intranasal pressure and nasal secretions (Kobayashi and Ito, 1995). Concomitant exposures to histamine indicated an additive effect on increasing nasal resistance. The authors concluded that diesel exhaust particles are potent in increasing nasal airway resistance and augmenting nasal airway resistance and nasal secretions in conjunction with histamine.

Intratracheal instillation of diesel particulates in mice was shown to result in increased lung water content, whereas administration of activated charcoal particles (Norit) had no effect (Ichinose *et al*., 1995). Bronchoalveolar lavage fluid (BALF) from mice treated with diesel particles had a significantly higher content of neutrophils compared with mice treated with Norit. The inflammation observed in the mice treated with diesel was preceded histologically by the detachment of capillary endothelial cells from their basement membrane and necrosis. Desquamation of type I pneumocytes was observed 6 hours after instillation of diesel particles by electron microscopy. Activated charcoal exposure resulted in an increase in alveolar macrophage content and a slight increase in neutrophil in the air spaces. No effect of Norit was seen in the bronchioles.

Ulfvarson *et al*. (1995) had also shown inflammatory airway changes, especially in the bronchiolar epithelium in rabbits exposed by inhalation to filtered or unfiltered diesel exhaust for 24 hours, with varying effects on lung function, including increased airway resistance, and increased or decreased compliance for unfiltered and filtered exhaust, respectively.

Another investigator, Chen (1986), evaluated the relative ability of particulate matter extract from the exhaust of a light-duty diesel engine and of pure benzo(a)pyrene (BaP) to induce aryl hydrocarbon hydroxylase (AHH) in lung and liver. Administered intratracheally one time, the DE extract caused a mild induction of lung AHH activity (112%). The increase in AHH activity from BaP exposure was approximately 4-fold. BaP was therefore determined to be a much more potent inducer of AHH activity than diesel particle extract. AHH induction is not, by itself, an adverse effect, but is an indicator of bioavailability of toxic PAH compounds.

4.1.3.2 SHORT-TERM EXPOSURE (UP TO 1 MONTH)

Several studies have examined the effects of repeated exposure to DE from light-duty engines for durations up to 1 month. These studies are summarized in Table 4.2. Inhalation exposure concentrations ranged from 0.46 to 6 mg/m^3 DE.

Using the lowest exposure concentration of pure DE, 3.3 mg/m^3, Henderson (1988a) exposed rats and mice by inhalation for 7 hour/day, 5 day/week, for up to 17 days. Prostaglandin F2α and leukotriene B4, two mediators of inflammation, were significantly elevated in bronchoalveolar lavage fluid after 2 days of exposure. Levels decreased to near control levels by day 17, indicating a possible adaptive response. The rats appeared to be more sensitive to the effects of DE than mice. This study suggests a mechanism of inflammation for this short-term exposure.

Following inhalation of 6 mg/m^3, Wright (1986) reported significant increases in the rate of DNA synthesis and in lung lipid metabolism in rats, and Pepelko and Peirano (1983) reported increased susceptibility to bacterial infection in mice.

Inhaling a mixture of DE particles at 0.46 mg/m^3, together with a slightly greater concentration of acids, induced significant decreases in phagocytic activity in rats (Prasad *et al*., 1988). The authors did not include an acid-only control for comparison.

Chen *et al*. (1981), using high concentrations with intraperitoneal injection, reported increased AHH activity in lung and liver of rats. Exposure to inhaled diesel particulates at 1.5 mg/m^3 for 6 months resulted in decreased AHH activity.

Together, these results are consistent with an acute or subacute inflammatory process at sufficiently high concentrations of DE.

The immunological effects of DE in rodents is discussed in a separate section (Section 4.3).

4.1.3.3 SUBCHRONIC EXPOSURE (1 TO 6 MONTHS)

Several groups of investigators have evaluated the effects of subchronic inhalation exposure on the lung. These studies are summarized in Table 4.3.

All the light-duty diesel exhaust studies, except one, utilized relatively high exposure levels (i.e. equal to or greater than 6 mg/m^3 DE). Barnhart *et al*. (1981), utilizing guinea pigs as an animal model, examined the effects of the lowest levels of DE. Animals were exposed to 0, 0.75 or 1.5 mg/m^3 DE for 20 hour/day, 5.5 days/week for up to 6 months. Exposure to 0.75 mg/m^3 resulted in an increase in the size and number of epithelial type II cells which subsequently produced an increase in the thickness of the air-blood barrier. In general, the 1.5 mg/m^3 exposure level produced similar but more severe effects than those observed in the 0.75 mg/m^3 exposure group.

Other subchronic studies of health effects of exhaust from light-duty diesel engine produced a variety of results at higher concentrations. At 6 mg/m^3 ,8 hours/day, 7 days/week for 3 months, Pepelko and Peirano (1983) reported a significant decrease of spontaneous locomotor activity level in exposed rats, and enhanced susceptibility to infection by Streptococcus pyogenes in mice. Using the same exposure regimen but at two doses (6 and 12 mg/ m^3) and for 6 months, the same authors reported in exposed Chinese hamsters a dose-dependent increase of lung weight and a decrease in lung function (carbon monoxide diffusing capacity) along with hyperplasia of alveolar Type II cells, resulting in a thickening of the alveolar epithelium. The exposed rats and mice also had large increases in lung protein content coupled with a decrease in total protein synthesis, suggesting the likelihood of pulmonary fibrosis development. Chinese hamsters exposed to 6.4 mg/m^3 for 6 months exhibited an increase in lung weight with a decrease in vital capacity, residual volume and carbon monoxide diffusing capacity, suggesting possible emphysematous changes (Vinegar *et al*. 1981). These studies are generally consistent with inflammatory changes in the lung.

Lewis *et al*. (1986), Hahon *et al*. (1985) and Rabovsky *et al*. (1986) evaluated the effects of heavy-duty diesel exhaust (HDE) on lung infectivity in CD-1 mice. Animals were exposed to 2 mg/m^3 DE for 7 hours/day, 5 days/week for 1, 3, or 6 months and then inoculated with influenza virus. The 3 and 6 month exposed animals exhibited virus-induced lung consolidation, higher peaks of virus multiplication, and lower levels of interferon in both lung and serum. These results indicate an increased level of susceptibility to infection following diesel exhaust exposure.

Adjusting the exposures for hours per week gives lowest observed effects levels of approximately 2 mg/m^3 for all four of the light-duty engine studies with only one non-zero exposure. The fifth study, which had two non-zero exposures, gave a lowest observed effect level of 0.5 mg/m^3.

Adjusting the exposure for hours per week gives lowest observed effects levels in the range 0.4 to 0.5 mg/m^3 in all of the experiments with heavy-duty engines and one with light-duty engines.

4.1.3.4. CHRONIC EXPOSURE (>6 MONTHS)

Ishinishi *et al*. (1986a, 1988) used electron microscopy to evaluate the morphological effects of 0.1, 0.4, 1 or 2 mg/m^3 light-duty diesel exhaust and 0.4, 1, 2, or 4 mg/m^3 heavy-duty diesel exhaust in rats. Animals were exposed 16 hours/day, 6 days/week for 30 months. Morphological changes were clearly observed in animals exposed to 1 and 2 mg/m^3 but not at 0.4 mg/m^3. The changes observed included: proliferating type II epithelial cells over the alveolar walls which developed into glandular metaplastic foci; marked infiltration of macrophages, plasma cells and fibroblasts into the alveolar septa; and local increases in collagen fibers resulting in sporadic thickening of the alveolar wall.

Nikula and associates (1995) found non-neoplastic lesions in F344 rats related to the lung burden of retained particles after 18 or 24 months. In their study, diesel exhaust exposures (2.44 or 6.33 mg/m^3) resulted in significantly increased lung weight from particulate burden, compared with the same particulate air concentration of carbon black. Diesel exhaust was, therefore, a more potent inducer of non-neoplastic lung lesions than carbon black particles. For most non-neoplastic lesions measured, including alveolar macrophage hyperplasia, alveolar epithelial hyperplasia, chronic-active inflammation, septal fibrosis, alveolar proteinosis, bronchiolar-alveolar metaplasia, and focal fibrosis with epithelial hyperplasia, diesel exhaust exposure resulted in increased incidence and severity of effect.

Lewis *et al*. (1986, 1989) exposed cynomolgus monkeys (15 per group) to 0 or 2 mg/m^3 diesel exhaust for 7 hours/day, 5 days/week, for 24 months in order to examine the effects of diesel exhaust on pulmonary function. Additional groups of 15 monkeys were exposed to either coal dust alone or in combination with the diesel exhaust. Results of the study included a significant but non-dose-dependent increase in mild pulmonary acute and chronic inflammatory responses resulting from the various particulate exposures. In addition, the study showed that monkeys exposed to diesel exhaust exhibited reduced pulmonary flow (obstructive disease), but not reduced lung volume (restrictive disease). The obstructive responses began to occur significantly after 12 months. A NOAEL for pulmonary effects of diesel exhaust exposure was not determined. In parallel studies using rats, these researchers found that maximal contractile responses in excised rat tracheal tissue were increased with each of the experimental exposures, compared with tissue from control rats.

The Lewis *et al*. (1986) study illustrates the utility of non-human primates for studying the non-cancer effects of diesel exhaust. However, the study included only a single concentration level, therefore a dose-response relationship could not be determined. In addition, there were significant effects observed at the one concentration, therefore no NOAEL was apparent. The relative utility of the monkey model as an alternative to the rat model for the effects of diesel exhaust in humans cannot be determined from the limited data presented in the Lewis *et al*. (1986) study.

Strom *et al*. (1984) exposed rats to diluted diesel exhaust for six months and one year, in order to determine effects on the pulmonary phagocytic responses. They used three atmospheric concentrations, 0.25, 0.75 and 1.5 mg/m^3. They reported that responses at the lowest exposure

concentration were similar to controls. At the two higher concentrations, the lavaged alveolar macrophages had increased volumes, polymorphonuclear leukocytes were present, and at one year lymphocytes were observed. Measurements of protein, beta-glucuronidase activity, and acid phosphatase activity were all elevated in the cells from animals with the two higher exposure concentrations.

Nagai *et al*. (1996) found that a 2-year exposure of guinea pigs to diesel exhaust with or without particulates for 16 hours/day, 6 days/week resulted in alveolar destruction in the form of punctate holes in the alveolar walls. The lowest concentration tested resulted in formation of alveolar holes (0.21 mg/m^3 as diesel particles). No NOAEL was observed in the study. Higher concentrations and increasing durations produced a greater number and size of alveolar holes. The authors measured the holes with electron microscopy and verified that the holes were not secondary to increasing alveolar airspace. The holes (or fenestrae) were considered distinct from the normal "pores of Kohn" which are found in healthy alveoli, due to their increased number and diameter. Controls and treated animals had the same number of alveoli and the same alveolar size. Pulmonary function was not measured. The reversibility of these holes and the extent of the functional damage to the lung were not investigated, but the presence of alveolar holes is correlated with onset of emphysema (Kobzik and Schoen, 1994).

Navorro *et al*. (1981) had previously reported impairment of the ability to metabolize benzo(a)pyrene in rat pulmonary microsomes at 1.25 mg/m^3 LDE for 20 hour/day, 5.5 days/week for 1 year. They found no effect on the enzyme activity of liver microsomes.

Oberdorster *et al*. (1992) reported that the influence of particles on inflammatory responses in the lung were a function of particle surface area and interstitial access. In their studies on male rats, ultrafine particles (< 20 nm) were more potent than larger particles (< 200 nm) in inducing pulmonary inflammatory events.

A large number of chronic animal studies are described in Appendix A, which is the U.S.EPA (1993) documentation for the Reference Concentration for Chronic Inhalation Exposure (RFE) for diesel engine emissions. Additional studies are described in Table 4-4 at the end of this section.

4.1.3.5 SUMMARY OF ANIMAL RESPIRATORY HEALTH EFFECTS

The inhalation or direct application of diesel into the respiratory tract of animals in acute and subchronic studies induced inflammatory airway changes, lung function changes, and increased susceptibility of exposed animals to lung infection. The morphological effects observed in the lungs of animals in chronic inhalation exposures are mainly related to chronic inflammatory responses. These changes include thickening of the alveolar epithelium, increase in lung weight, infiltration of macrophages, fibroblasts and proteins into the alveolar septa, and glandular metaplasia. The use of the Ishinishi *et al*. (1988) study to derive the reference exposure level for diesel exhaust is discussed in Section 4.5.2.

4.2 REPRODUCTIVE AND DEVELOPMENTAL HEALTH EFFECTS

This section summarizes the literature on the reproductive and developmental effects of diesel exhaust exposure in animals. Details of these studies are presented in Table 4.5. There are no data available in the literature concerning such effects in humans (IARC, 1989).

Studies on induced heritable point mutations and sperm abnormalities following diesel exhaust exposure were negative in rats, mice, and monkeys, though sperm anomalies were noted in exposed hamsters. An observed decrease in the number of corpora lutea following diesel exhaust exposure in one study suggests potential impact on female reproduction in mice. Teratogenic effects were not observed in rabbits. Some evidence of neurobehavioral effects in rat pups is reported.

4.2.1 MALE REPRODUCTIVE STUDIES

Pepelko and Peirano (1983) observed evidence of heritable point mutations in the offspring of hybrid male mice that had been exposed by inhalation to diesel exhaust for 5 or 10 weeks (8 hours/day, 7 days/week to 6 mg/ m^3). In the dominant lethal test, inhalation exposure of male mice and rats to diesel exhaust did not induce detectable chromosome changes in germ cells (Pepelko and Peirano, 1983; Lewis *et al*., 1986; Callahan *et al*., 1986). There were no differences in the number of females pregnant, the number of implants, or the number of living embryos between the control and the diesel exhaust exposed groups.

The test for heritable translocations did not demonstrate differences in sterility or partial sterility in the male progeny from pre-exposure and post-exposure matings (Pepelko and Peirano, 1983). No effect on spermatogonial survival were observed between control and diesel exhaust exposed mice (Pepelko and Peirano, 1983). However, a 2.67-fold increase in sperm abnormalities was observed in Chinese hamsters exposed 8 hours daily for 6 months to 6 mg/m^3 diesel exhaust (Pereira *et al*., 1981b). Mice exposed to a similar exposure protocol did not exhibit an increase in the number of abnormal sperm (Pereira *et al*., 1981c). The investigators note, however, that the very high spontaneous rate of sperm shape abnormalities in this strain of mice could have masked any small positive effect.

Sperm abnormalities were detected in mice exposed to diesel exhaust particulate via intraperitoneal injection. The effect was most notable in the high exposure group (200 mg/kg) where the extent of the abnormalities was 8 times higher than the spontaneous level. A significant decrease in sperm count was also observed at the highest dose though testicular weight was not influenced (Quinto and DeMarinis, 1984). No significant changes were detected in number, motility, velocity or morphology of sperm from Cynomolgus male monkeys that had been exposed by inhalation for 7 hours per day, 5 days per week for 24 months to 2 mg/m^3 diesel exhaust (Lewis *et al*., 1986).

Fredricsson *et al*. (1993) reported a dose-dependent decrease in the percentage of mobile sperm and generalized motility impairment in human sperm cells exposed in vitro to extracts of diesel exhaust for several hours.

4.2.2 FEMALE REPRODUCTIVE STUDIES

No difference in reproductive performance as measured by litter size was observed between control female mice and mice exposed daily by inhalation to 6 mg/m^3 diesel exhaust for 8 weeks prior to mating (Pepelko and Peirano, 1983). Consequently, the investigators found no evidence of chromosome or cytotoxic effects in oocytes. The same investigators obtained negative results in the dominant lethal mutation test. The percentage of matings resulting in pregnancy, the number of live embryos, and implantation loss were not different between the controls and mice exposed by inhalation to diesel exhaust 7 weeks prior to mating. However, fewer corpora lutea were noted in the exposed group. In addition, the time interval from caging to copulation was significantly longer in the exposed animals as compared to the controls. The investigators showed that the observed decrease in corpora lutea was not merely a function of the delay in mating. Such effects on the corpora lutea would be consistent with alterations in ovulation and/or estrus cycle.

4.2.3 TERATOGENICITY STUDIES

Pepelko and Peirano (1983) reported two studies, one in rats and one in rabbits, to assess how inhalation exposure to DE during the critical period of gestation affects fetal development. Animals were exposed to 8 hours/day, 7 days/week to 6 mg/ m^3 during the critical period of gestation (days 5 - 16, rats; days 6 - 18; rabbits). One day prior to the projected parturition date, the dams were killed and all fetuses were removed and examined for external abnormalities. One third of the fetuses were further examined for gross internal anomalies while the remaining two thirds were examined for skeletal abnormalities. Reproductive parameters, including total number of fetuses (live and dead), resorptions, implants, and corpora lutea (see Table 4.5 for other variables investigated), were scored. The studies reported no significant differences between animals in the control and exposed groups of either species. Callahan *et al*. (1986) reported results of a study in rats exposed during gestation to tank-generated diesel exhaust which gave some indication that diesel exhaust is fetotoxic and/or results in delayed development. Sites of retarded ossification were noted in the vertebral columns, ribs and sternums of the fetuses of the exposed dams.

4.2.4 GENERATIONAL STUDIES

Pepelko and Peirano (1983) investigated reproductive performance in mice exposed by inhalation (8 hours/day, 7 days/week to 6 mg/m^3) to diesel exhaust from 100 days prior to breeding through maturity of the F_2 generation. The authors concluded that treatment-related effects were slight. Although some differences in organ and body weights were noted in the F_2 generation, overall fertility and survival rates were not significantly different between exposed and control animals. A significant increase in lung weight of the exposed animals was observed in all F_0 and F_1 breeders, F_1 unmated mice and F_2 generation male pups. The increase in lung weight was seen in conjunction with a gross pathological diagnosis of anthracosis.

Callahan *et al*.(1986) exposed male and female rats to 15- or 60-min, 6 mg/m^3 of diesel exhaust in connection with time of mating in order to determine effect on mating success, period of

gestation, delivery, and care of pups. The study did not detect any effect on these gross endpoints.

4.2.5 DEVELOPMENTAL STUDIES

Mauderly *et al*. (1987a) exposed rats from conception to 6 months of age to diesel exhaust in an attempt to assess any effects on lung development. Although the investigators concluded that general lung development was not affected by exposure, alterations in airway fluid constituents and lung enzymes were observed. It was also noted that effects in these young rats were much less severe than those seen in adults exposed to the same diesel exhaust levels.

In another study investigating neurobehavioral effects, Laurie *et al*. (1981a, in Pepelko and Peirano, 1983) exposed two groups of rat pups to 6 mg/m^3 diesel exhaust from either day 1 to day 17 of age for 20 hours per day or from day 1 to day 21, 28 or 42 of age for 8 hours per day. The results of this neurobehavioral study demonstrated depressed spontaneous locomotor activity (using standard running wheels) in the exposed animals. These effects were less pronounced in those animals exposed for only 8 hours per day to diesel exhaust. Operant conditioning, as measured by acquisition of the bar pressing task, in 15 month old animals exposed as neonates for 20 hours per day was significantly delayed relative to the controls.

The same investigators examined neurophysiological alterations in diesel exhaust exposed neonates as measured by changes in somatosensory and visual evoked potentials (SEPs and VEPs, respectively) (Laurie and Boyes, 1981b). Rat pups were exposed to air or 6 mg/m^3 diesel exhaust from birth to 7, 14, 21 or 28 days of age. A significant change in the latencies of SEPs and VEPs relative to the controls was noted only in the 1 to 14 day exposure group. The investigators pointed out that the most rapid rate of neural growth occurs at about 14 days of age and that a toxic effect would be most pronounced in that critical time period.

4.2.6 SUMMARY OF REPRODUCTIVE/DEVELOPMENTAL EFFECTS

Inhalation of diesel exhaust did not induce heritable point mutations or translocations in male rodents, nor dominant lethals in animals of either sex. Exposure via inhalation did not induce sperm abnormalities nor affect spermatogonial survival in mice and monkeys though sperm anomalies were observed in hamsters.

Data of the effects of diesel exhaust exposure on female reproductive capacity are limited but potential effects on corpora lutea and mating period have been suggested in laboratory rodents.

No teratogenic effects of diesel exhaust exposure were shown in rabbits. Delayed ossification of the thoracic region has been noted in rats following exposure to very high exposure levels. Exposure to diesel exhaust during the neonatal developmental period of rodents induces neurobehavioral and neurophysiological effects, but does not affect general lung development. Other organ systems have not been evaluated.

Generational studies conducted in rodents revealed that inhalation exposure to diesel exhaust causes increases in lung weight in all generations examined. Evaluation of other parameters produced inconclusive results.

The available literature does not provide sufficient information to determine whether or not diesel exhaust exposure induces reproductive, developmental or teratogenic effects in humans.

4.3 IMMUNOLOGICAL EFFECTS

There are a number of review articles which postulate that air pollution, particularly diesel exhaust particles, plays a role in the increasing prevalence of asthma and other allergic respiratory disease (Albright and Goldstein, 1996; Peterson and Saxon, 1996; Devalia *et al*., 1997). The discovery of the role of diesel particulates and their PAH fraction in augmentation of allergic responses to specific antigens in humans and animals is relatively new, and many of the studies discussed below are comparatively recent (Peterson and Saxon, 1996; Diaz-Sanchez, 1997).

Diesel exhaust exposure can result in measurable increases in IgE and IgG antibody production, perturbed cytokine regulation, localized inflammation and eosinophilic infiltration in lung and respiratory tract tissues.

4.3.1 HUMAN STUDIES

A number of recent studies indicate that diesel exhaust particles (DEP) could induce immunological allergic reactions as well as localized inflammatory responses in humans (Diaz-Sanchez *et al*., 1994, 1996, 1997; Terada *et al*., 1997; Takenaka *et al*., 1995). Diaz-Sanchez *et al*. (1994) found that intranasal instillation of 0.30 mg DEP (0.15 mg per nostril in 5 ml saline) to 11 volunteer subjects resulted in a significant rise in localized (nasal) IgE antibody production and mRNA that coded for IgE proteins. The elevation in IgE was specific as no concomitant rise in levels of IgA, IgG, IgM, or albumin were observed. Extending this study to T cells macrophages and epithelial cells, Diaz-Sanchez *et al*. (1996) showed that the mRNAs for the pro-inflammatory cytokines, interleukin-4 (IL-4), IL-6, IL-10, and interferon-gamma, were all significantly increased in human volunteer subjects after challenge with 0.2 mg DEP.

Several studies have suggested that DEP may act as an adjuvant for pollen allergy. Diaz-Sanchez *et al*. (1997) found that in allergic human subjects, nasal IgE antibody response to ragweed allergen was significantly elevated by co-exposure to DEP over ragweed alone. DEP may also influence antigen presentation or may act as a vector for submicron fragments of pollen grains which would otherwise be too small to be deposited in human airways (Knox *et al*., 1997). It was suggested that asthma outbreaks associated with thunderstorms might be triggered by exposure to pollen allergens carried into the lungs by DEP (Frew and Salvi, 1997). In Japan, Ishizaki *et al*. (1987) reported that residents living along intercity main streets lined with old cedar trees and with heavy traffic had high incidence of cedar pollenosis compared with individuals living in cedar forest areas with less traffic. Pollen counts in both areas were identical. The number of diesel cars in use in Japan increased from 15,000 in 1951 to more than

5 million in 1983 (Muranaka *et al*., 1986). It was postulated that the increased air pollution caused by diesel vehicles was responsible for the higher incidence of cedar pollenosis.

In in vitro experiments human extract of PAHs from DEPs (PAH-DEP) enhanced the production of IgE in purified human B cells prepared from blood mononuclear or tonsil cells (Takenaka *et al*., 1995) and by 2C4/F3, a human Epstein-Barr virus transformed IgE producing B cell line (Tsien *et al*., 1997). These studies were used to investigate the mechanism of PAH-DEP on IgE production. Airway epithelial cells which probably play a pivotal role in the pathogenesis of airway disease, are often used in mechanistic studies of air pollutant-induced airway diseases. DEP increased the synthesis and release of pro-inflammatory mediators, including eicosanoids, cytokines, and adhesion molecules by cultured human bronchial epithelial cells (Devalia *et al*., 1997). Terada *et al*., (1997) found that DEP extracts enhanced human eosinophil adhesion to nasal epithelial cells and caused eosinophil degranulation. Since eosinophil degranulation is known to release mediators and proteins known to play important roles in the development of nasal allergy (Terada *et al*., 1992), the authors concluded that DEP can promote nasal eosinophil-mediated hypersensitivity.

Although the increased worldwide prevalence of asthma and allergic diseases has often been associated with the increased use of diesel fuel (Peterson and Saxon, 1996; Rusznak *et al*., 1994), there is only one report which directly relates the development of asthma with exposure to diesel exhaust. Wade and Newman (1993) reported three railroad workers developing asthma following excessive exposure to locomotive diesel emissions. Asthma diagnosis was based on various symptoms such as pulmonary function tests, and measurement of airways hyperactivity to methacholine or exercise. These workers had no previous history of asthma or other respiratory disease and were not current smokers. The sensitizing and irritant properties of diesel oil and their indicator dyes were investigated by Fisher and Bjarnason (1996). Tests on human subjects failed to demonstrate contact allergy or contact urticaria to the dyes or dyed diesel oils.

4.3.2 ANIMAL STUDIES

Early studies yielded mixed results for immunological effects of diesel in rodents, but these studies did not specifically focus on allergic immune responses. For example, Bice *et al*. (1985) showed that rats immunized with sheep erythrocytes (SRBC) and exposed to 3500, or 7000 $\mu g/m^3$ diesel exhaust for up to 24 months had significantly elevated anti-SRBC IgM antibody forming cells in the lung associated lymph nodes and spleen. The number of anti-SRBC IgM antibody forming cells was not significantly increased in mice similarly exposed. Total antibody production (IgM. IgG, or IgA) was not significantly altered in either rats or mice. SRBC does not elicit an IgE response and is not a model for allergenicity. The increase in total lymphoid cellularity was not explained, and the authors concluded a minimal effect of DEP on immune function.

Muranaka *et al*. (1986) found that the primary IgE antibody responses in BDF1 mice immunized with a single or repeated intraperitoneal injection of ovalbumin (OA) mixed with DEP or Japanese cedar pollen (JCP) mixed with DEP were significantly higher than those in animals

immunized with the antigens alone (OA or JCP, respectively). Similar results were obtained by Takafuji *et al*. (1987) using the same protocol but applying the DEP (1 μg) intranasally in mice every 3 weeks for 15 weeks. These authors concluded that DEP is an adjuvant for IgE antibody production. To investigate further the effects of DEP on IgE antibody production, Fujimaki *et al*. (1994) injected BALB/c mice intratracheally with DEP (0.3 mg) mixed with an antigen, OA or JCP. IL-4 levels in the culture supernatants of mediastinal lymph node cells were significantly increased in mice injected with DEP and OA, and DEP and JCP than those from mice injected with the antigen alone. Similar results were obtained in experiments performed intranasally (5μg DEP) and by inhalation (0, 3 and 6 mg/m^3 DEP for three weeks) (Fujimaki *et al*., 1995, 1997). They concluded that DEP given by the three routes (intratracheally, intranasally or by inhalation) affects antigen-specific IgE antibody responses and enhances cytokine production. The adjuvant effect of DEP and carbon black (CB) with the antigen OA was studied in BALB/c mice using the popliteal lymph node assay (Lovik *et al*., 1997). A suspension of 5 mg/ml of DEP or CB was used for footpad inoculation. The OA-specific IgE response was increased in mice receiving OA and DEP or OA and CB when compared to the response in mice receiving OA alone. Both DEP and CB had a significant adjuvant effect on the local immune-mediated inflammatory response and on the systemic specific IgE response to the allergen OA.

Kobayashi *et al*. (1997) used a rhinitis model of guinea pig to investigate whether short-term exposure (3-hr) to DEP induces nasal mucosal hyperresponsiveness to histamine. Animals were exposed to filtered air or to 1 and 3.2 mg/m^3 DEP. Using sneezing frequency, nasal secretion, and intranasal airway resistance induced by histamine as indices of rhinorrhea and nasal congestion, the authors concluded that short-term exposure to DEP potently induces nasal hyperresponsiveness to histamine.

To study the effect of DEP on the production of granulocyte-macrophage colony stimulating factor (GM-CSF), DEP (dose not given) was added to a culture of BEAS-2B cells (a bronchial epithelial cell line). A "dose-dependent" increase in production of GM-CSF was observed (Miyamoto, 1997). DEP administered transnasally to mice every 2 days for 2 weeks exhibited increased airway responsiveness to acetylcholine compared to untreated controls. This response was completely inhibited by goat anti-mouse GM-CSF antibody administered transnasally but not subcutaneously. It is postulated that GM-CSF production causes airway inflammation and may play a role in airway hyperreactivity and asthma (Miyamoto, 1997). Yang *et al*. (1997) found that DEP or methanol extracts of DEP increased interleukin-1 (IL-1) production by rat alveolar macrophages (AM) in vitro, but suppressed IL-1 and tumor necrosis factor (TNF-alpha) production by DEP-pretreated AM in response to bacterial lipopolysaccharide (LPS). They concluded that the suppressive response of DEP-pretreated AM to LPS stimulation may contribute to the impairment of pulmonary defense system after DEP exposure.

Sagai *et al*. (1996) studied the "asthma-like symptoms" induced in mice instilled intratracheally (once/week for 16 weeks) with DEP (0.1-0.2 mg). There was a marked infiltration of eosinophils in the submucosa of the proximal bronchi and medium bronchioles, proliferation of goblet cells, increased mucus secretion, and increased respiratory resistance and airway constriction in mice treated with DEP. These responses were significantly suppressed by pretreatment with

polyethylene glycol-conjugated superoxide dismutase (PEG-SOD). These authors concluded that airway inflammation caused by DEP could be due to superoxides and hydroxy radicals produced by DEP because the toxic actions observed was reduced by pretreatment with PEG-SOD. Using lung protein analyses and bronchoalveolar lavage supernatants (BALS), Takano *et al*. (1997) reported the modulation of immune cytokines following intratracheal instillation of DEP (100 μg in 0.1 ml of phosphate-buffered saline, weekly for 6 weeks) in mice. Interleukin-5 levels were increased, as were IL-4, IL-2, and GM-CSF compared to control levels. The inflammatory cells present in the BALS, such as neutrophils, macrophages, eosinophils, and lymphocytes, all were greatly increased when DEP was administered in conjunction with OA antigen, compared to controls or either treatment alone. In addition, DEP exhibited adjuvant activity for antigen-specific IgG and IgE. Similarly, Ichinose *et al*. (1997a) found that different strains of mice when exposed intratracheally to DEP (0.1 or 0.2 mg in 0.1 ml saline buffer) exhibited enhanced allergic airway inflammation, measured by eosinophil infiltration, IgE, and IgG production in response to OA. According to the authors, DEP may play a role in the mechanisms involved in allergic asthma.

4.3.3 SUMMARY OF IMMUNOLOGICAL EFFECTS

It is apparent from the recent allergy and immunology literature that diesel exhaust exposure can result in measurable increases in IgE and IgG antibody production, perturbed immunological cytokine regulation, localized inflammation and eosinophilic infiltration in lung and respiratory tract tissues, particularly when the exposure accompanies other known respiratory allergens. In human subjects and in human cells, DEP stimulated IgE antibody production and increased mRNA for the pro-inflammatory cytokines. Co-exposure to DEP and ragweed pollen was reported to significantly enhanced the IgE antibody response relative to ragweed pollen alone. DEP also enhanced the IgE antibody and cytokine production response to ovalbumin and Japanese cedar pollen in animal models and increased nasal hyperresponsiveness to histamines. There is some evidence that production of reactive oxygen species may be involved in the asthma-like symptoms produced in mice by DEP exposure.

Although none of the studies evaluated have been designed to yield quantitative estimates of diesel particle concentrations for the purposes of determining a reference exposure level, the potential relevance of these endpoints to public health is very high, due to reports of large numbers of individuals with respiratory allergies and asthma in urban areas (Burney *et al*., 1990; Corbo *et al*., 1993; Emanuel, 1988; Strachan and Anderson, 1992; Frew and Salvi, 1997).

4.4 APPROACHES USED IN ESTABLISHING NON-CANCER HEALTH LEVELS

The establishment of health levels by the U.S. EPA, WHO and OEHHA are all based on the chronic rat study by Ishinishi *et al*. (1988) and other studies. The U.S.EPA used the NOAEL/LOAEL approach where the NOAEL based on a human equivalent concentration from an observed NOAEL obtained from Ishinishi *et al*. study, was divided by an uncertainty factor to obtain the RfC of 5 $\mu g/m^3$. A similar approach, also based on the Ishinishi *et al*. study, was used

by the WHO to obtain a guidance value of 5.6 $\mu g/m^3$. In addition, the WHO also obtained benchmark concentrations (BC_{05}), from 2 to 3 $\mu g/m^3$, using the Weibull model on several other animal data sets. The OEHHA performed benchmark concentration calculations using the log-normal probit and Weibull models on the Ishinishi *et al*. (1988) data and obtained values ranging from 2 to 21 $\mu g/m^3$. The best point estimate within this range is 5 $\mu g/m^3$, which the OEHHA is recommending as the chronic REL. This level is similar to the U.S. EPA's RfC.

4.4.1 THE U.S.EPA RFC

The documentation of the U.S.EPA RfC is found in Appendix A. Appendix A indicates a lowest observed adverse effects level (LOAEL) for diesel exhaust of 0.3 mg/m^3, based on a human equivalent concentration from an observed LOAEL of 0.96 mg/m^3 in the Ishinishi *et al*. (1988) study (see Table 4.6). The no observed adverse effects level (NOAEL) in that study was 0.46 mg/m^3. These data were used by U.S.EPA to derive the non-cancer RfC for diesel. The human equivalent concentration (HEC) to the NOAEL using a dosimetric adjustment for physiological differences for particle deposition in the lung (Yu *et al.*, 1990) is 0.16 mg/m^3. The Yu and Yoon model used by U.S.EPA apparently adjusts for the discontinuous exposures from the Ishinishi *et al*. (1988) study, since no time-adjustment is included in the calculation of the RfC.

The U.S.EPA (1993) divided the NOAEL of 0.16 mg/m^3 by an uncertainty factor of 30 to obtain the reference concentration (RfC) of 5 $\mu g/m^3$. The uncertainty factor reflects a factor of 10 to protect sensitive individuals. A factor of 3, instead of the customary value of 10, was used to adjust for interspecies extrapolation because dosimetric adjustments based on a particle deposition and retention model were applied. There is scientific support for the inclusion of an uncertainty factor for species differences with diesel exhaust exposure. It has been reported that there are not comparable studies as yet in the human that are sufficient to compare the macrophage particle interaction in the human with the macrophage particle interaction in the rat (Dungworth, oral testimony 1994). It is therefore not presently possible to ignore potential interspecies differences in toxicological responses resulting from inflammation and hyperplasia following diesel exhaust inhalation. In addition, the recent studies in humans (Diaz-Sanchez *et al*., 1994; Takenaka *et al*., 1995; Wade and Newman, 1993) showing allergic and asthmatic reactions to diesel exhaust indicate potential susceptibility of sensitive subpopulations to diesel exhaust, and further support the retention of an interspecies uncertainty factor, since these effects have not been observed in the rat. Potential interspecies differences in toxicological responses resulting from immunological effects following exposure to diesel exhaust should not be ignored. The available data appear to support this position and argue for retention of an interspecies uncertainty factor for pulmonary responses to diesel. The resulting RfC is thus an estimate (with uncertainty spanning perhaps an order of magnitude) of a safe daily inhalation exposure for the human population. This exposure is expected to be without an appreciable risk of deleterious noncarcinogenic effects during a lifetime.

4.4.2 WORLD HEALTH ORGANIZATION (WHO) ANALYSES

Similar to the U.S. EPA RfC document, the World Health Organization (WHO, 1994) calculated threshold concentrations for human health effects of diesel exhaust based on the Ishinishi *et al*. study and other data. Also presented in the WHO (1994) document are various benchmark concentrations using the Weibull model on several data sets. These approaches are presented below and the resulting analyses by WHO are shown in Table 4.7.

Several other studies indicate lower thresholds (based on benchmark analyses) for different endpoints. The Creutzenberg *et al*. (1990) study in rats indicates that impaired lung clearance and neutrophil infiltration occurred at 0.45 mg/m^3. A study by Nikula *et al*. (1995) indicates that an 18-month exposure of female rats to 2.44 mg/m^3 diesel exhaust resulted in 100% incidence of alveolar macrophage and epithelial hyperplasia. NOAELs were not determined in the above 2 studies.

Benchmark concentration (BC_{05}) calculations based on deep-lung clearance and alveolar inflammation measured by neutrophil infiltration yield threshold estimates for the onset of non-cancer adverse effects in rats from 2 to 3 μg/m^3 (Creutzenberg *et al*., 1990; Henderson *et al*., 1988, cited in World Health Organization, 1994). Table 4.7 illustrates the proposed guidance values from WHO (1994) together with their scientific bases. These estimates also consider the interspecies dosimetry adjustments of Yu *et al.* (1990) used by U.S.EPA in the calculation of the RfC. As can be seen from Table 4.7, the absence of dosimetric adjustments results in guidance concentrations (0.9 to 6.3 μg/m^3) that form a range consistent with the U.S.EPA RfC. However, these estimates contain considerable uncertainty. From the results of the WHO (1994) analysis, pulmonary alveolar inflammation and impaired deep-lung clearance appear to be the most sensitive endpoints in rats. The Ishinishi *et al*. (1988) study was selected by U.S.EPA as the best overall study for determination of the RfC after considering sample size, sensitivity of endpoint, clear dose-response, and consideration of particle size through use of 2 different grades of diesel exhaust.

Continuity of exposure is apparently an important factor in determining severity of the outcome, as illustrated when comparing the results of Creutzenberg *et al*. (1990) to the results of Griffis *et al*. (1983) for rats. The results by Creutzenberg *et al*. mentioned above were significant at lower concentrations due to the longer duration of exposure each day (19 hours/day vs. 7 hours/day).

4.4.3 OEHHA ANALYSES

For comparison with the traditional NOAEL approach given by U.S.EPA, and the benchmark concentrations derived by the WHO, OEHHA performed benchmark concentration calculations using log-normal probit and Weibull models with the female rat data from Ishinishi *et al*. (1988). A benchmark concentration has greater precision than a NOAEL at estimating a true threshold response in the animal population. The result of using this more precise methodology is a reduction in the total uncertainty involved in extrapolating to a threshold of response for

sensitive individuals. OEHHA proposes that the typical 10-fold uncertainty factor between interspecies differences be reduced to 3-fold which is consistent with U.S. EPA practices.

In the Ishinishi *et al*. study, exposure duration was adjusted for continuity (16 hours/day, 6 days/week). An uncertainty factor of 30 was used; 10 for sensitive individuals and 3 for species dosimetry and response differences. Heavy duty diesel was determined to be more potent than the LD diesel for induction of hyperplastic lesions in rats using the log-normal probit analysis. Female rat data were suitable for the analysis, while male only, or combined male and female data were not. The dose-response relationship of diesel-induced lung hyperplasia in the males exposed to HD was not well modeled by the log-normal probit relationship, particularly at extrapolated low doses. For example, the 95% lower confidence limit on the MLE_{01} using the male HD data was 2.3×10^{-3} $\mu g/m^3$, or a factor of 4.4×10^5 below the MLE. This is partially due to the shallow dose-response slope from the male rat data. Furthermore, a test for the acceptance of combining data sets using maximum likelihood estimates from the log-normal analysis indicated that the male and female data sets should not be combined (Stiteler *et al*., 1993). The test for combining the data sets is shown below:

Data Set	Maximum Log Likelihood
Combined	-153.961
Female	-85.129
Male	-65.297

The natural logarithm of the Generalized Likelihood Ratio (GLR) is
[-153.961 - (-85.129 + -65.297)] = -3.535.

The likelihood ratio test statistic is calculated as
-2 (ln GLR) = 7.07

The chi-square test for one degree of freedom results in p = 0.008. Therefore the datasets should not be combined.

For the above reasons, the female rat HD diesel data were used to determine the benchmark concentration for diesel exhaust. Similar sex-dependent responses were observed in both the light and heavy duty diesel experiments.

Consonant with U.S.EPA and the World Health Organization, the OEHHA staff acknowledge a reasonable range of reference concentrations derived from the Ishinishi *et al*. (1988) study from 2 to 21 $\mu g/m^3$. Table 4.8 illustrates the resulting benchmark concentration calculations for diesel exhaust, based on the Ishinishi *et al*. (1988) study. The best point estimate within this range recommended by OEHHA is 5 $\mu g/m^3$ (U.S.EPA RfC, 1993; see Appendix A).

4.5 Existing U.S.EPA $PM_{2.5}$ Standard

The U.S.EPA has proposed an annual average standard for airborne particulate matter less than 2.5 microns ($PM_{2.5}$) of 15 $\mu g/m^3$ (U.S.EPA, 1996). The new standards are proposed to protect against a wide range of PM-related health effects, including premature mortality; increased hospitalizations and emergency room visits; increased respiratory symptoms and disease; decreased lung function; and alterations in lung tissue and respiratory defense mechanisms. Of the several dozen epidemiological studies conducted between 1988 and 1996, most support the proposal for more stringent standards.

Any reference exposure level for diesel exhaust must be considered within the framework of the standard for particulate air pollution. Diesel exhaust particles are fine, falling typically below 1 micron in diameter. Increasingly, fine suspended particulates are being ascribed the greatest potency in inducing particle-related health effects (U.S.EPA, 1996). Therefore, although OEHHA has determined a range of concentrations of diesel particles (2 - 21 $\mu g/m^3$) that would form a valid REL, any REL derived for diesel particles should not exceed the federal standard for fine particulates (15 $\mu g/m^3$). The U.S.EPA RfC for diesel exhaust of 5 $\mu g/m^3$ is consistent with this limitation.

4.6 CONCLUSION

The human epidemiology, case study, and clinical laboratory data indicate that diesel exhaust particles likely cause or aggravate a variety of adverse non-cancer respiratory effects in humans including impaired lung function, asthma and allergic reactions, in addition to general respiratory irritation. However, the literature on human health effects does not allow for a quantitative derivation of a reference exposure level for diesel exhaust at the current time. Therefore, it is appropriate to utilize data from experimental animals. The U.S.EPA has appropriately determined a reference exposure level based on the chronic rat study by Ishinishi *et al*. (1988) as the basis for the RfC for diesel exhaust. OEHHA recommends 5 $\mu g/m^3$ (U.S.EPA RfC, 1993; see Appendix A for full text) as a reference exposure level for diesel exhaust.

Table 4.1 Animal Non-Cancer Effects After Single-Dose Exposure to Light-Duty Diesel Engine Exhaust.

Exposure	Animal Species	Effects/Observations	Reference
Intratracheal Instillation			
One group of animals received either a single intratracheal instillation of diesel particulate extract (DPE) (3, 6, or 12 mg/kg) or benzo[a]pyrene (BaP) (0.15, 1, 3, 5 mg/kg) and was sacrificed 24 hours later. The second group received a single dose of either DPE (12 mg/kg) or BaP (5 mg/kg) and was sacrificed 12, 24, 48, 72, or 144 hours later.	Male Fischer 344 rats	3, 6 and 12 mg/kg DPE: Animals exhibited a dose-dependent increase in lung aryl hydrocarbon hydroxylase (AHH) activity, which peaked after 24 hours and returned to basal levels after 48 hours. No effect on liver AHH levels. 0.15-5 mg/kg BaP: Large, saturating dose-dependent increase in lung AHH activity, peaking 12 hours after administration and remaining elevated for seven days. Dose-dependent increase in liver AHH activity, which peaked 24 hours after administration and quickly returned to control levels.	Chen, 1986 J Appl Toxicol 6(4): 259-262
Groups examined for lung endothelial cell injury consisted of 4 animals per group, receiving 0.8 mg diesel exhaust particles (DEP) or activated carbon (Norit) and were sacrificed 0.5, 1, 2, 4, 6, 12, and 24 hours later. Controls received phosphate buffered saline (PBS) and were killed 1 hour later. Animals in groups of 5 were sacrificed 1, 2, 4, 6, 12, and 24 hours after administration of 0.4 or 0.8 mg DEP, or 0.8 mg Norit to determine water content in their lungs. Controls received 50 mm PBS and were sacrificed 1 hour later. Five animals per group were administered 0.4 mg DEP or Norit and were killed 4, 12, or 24 hours later for analysis of bronchial alveolar lavage fluid (BALF). Controls were sacrificed 24 hours after exposure to PBS.	Male ICR mice	Animals treated with 0.4 or 0.8 mg DEP had elevated ($p < 0.05$) lung water content compared with Norit control groups. Endothelial cell injury in the lung was significant ($p < 0.05$) in DEP-treated mice compared to Norit controls. Disruption and desquamation of type I pneumocytes occurred 6 hours after administration of DEP. An influx of neutrophils into the alveoli, intra-alveolar hemorrhage, perivascular edema and bronchiolar cell hypertrophy were seen in mice between 18 and 24 hours after DEP administration. Administration of Norit had no effect on bronchiolar cells or type I pneumocytes.	Ichinose *et al.*, 1995 Toxicology 99:153-167.
Groups of 4 guinea pigs were exposed to 0, 1.0, 10.0, or 20.0 mg/kg DEP suspension in buffered saline via intranasal cannula. Eighty minutes after DEP exposure, animals were exposed to histamine aerosol for 10 minutes. Intranasal pressure and nasal secretions were measured following histamine exposure.	Male Hartley Guinea Pigs	DEP exposures resulted in dose-dependent elevations in intranasal pressure with or without histamine exposure. Nasal secretion was also significantly elevated. DEP greatly increased the vascular permeability of the dorsal skin.	Kobayashi and Ito, 1995 Fundam Appl Toxicol 27:195-202.

Table 4.1 Animal Non-Cancer Effects After Single-Dose Exposure to Light-Duty Diesel Engine Exhaust (continued).

Exposure	Animal Species	Effects/Observations	Reference
Inhalation Exposure			
Study examined susceptibility to infection of mice challenged with aerosol pathogens. Animals were exposed to 0 or 6 mg/m^3 of diesel exhaust particulate (DEP) for 2 .or 6 hours, 8, 15, or 16 days (8 h/d, 7d/wk).. Immediately thereafter, animals were exposed to aerosol containing *Streptococcus pyogenes, Salmonella typhimurium,* or A/PR8-34 influenza virus. Source of DEP was from 2 Nissan CN 6-33 diesel engines.	Female CR/CD-1 mice	6 mg/m^3 DEP: Only Streptococcus pyogenes infectivity after 6 hour exposure was enhanced. Average mortality in controls was 6.7% vs. 31.7% in exposed animals.	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4) 253-306
Lung function (airway resistance and compliance, diffusing capacity and alveolar volume) and morphology was measured following a single 5-hour inhalation exposure to diesel exhaust with and without filter traps. Concentrations ranged from approximately 0.02 to 0.35 mg/cu m.	Male Chinchilla rabbits	Animals exposed to unfiltered exhaust exhibited increased neutrophil infiltration into alveolar interseptal regions and moderate inflammation of the large and small bronchi. A slight increase in inflammation was seen in both types of particle-filtered exhaust. Airway resistance was increased from exposure to either filtered or unfiltered exhaust. Lung compliance was correlated with changes in particle concentration, and with formaldehyde content (range: 0.052-0.10 mg/cu m), but not with nitrogen dioxide concentrations (range: 2.6-4.7 mg/cu m).	Ulfvarson *et al.*, 1995 Am J Ind Med 27:91-106

Table 4.2 Animal Non-Cancer Effects After Short-term (up to 1 month) Exposure to Diesel Exhaust.

Exposure	Animal Species	Effects/Observations	Reference
Light Duty Diesel Engine Inhalation Exposure			
Animals were placed in a chamber and received nose-only exposure to a combination of diesel exhaust particulate (DEP) and acid droplets (0.46 mg/m^3 DEP; 0.38 mg/m^3 HNO_3; 0.18 mg/m^3 H_2SO_4) or purified air, 5 h/d for 5 consecutive days. Animals were sacrificed 0, 1, or 2 days after exposure. Source of DEP was a single cylinder diesel engine burning Phillips No. 2 diesel control fuel.	Male Sprague-Dawley rats	0.46 mg/m^3DEP: Significant decrease in receptor surfaces for the immunoglobulin IgG and general phagocytic activity of pulmonary microphages. Surface receptor activity began to recover after the second day following exposure, while general phagocytic activity showed no signs of recovery over the study period.	Prasad *et al.*, 1988 J Toxicol and Environ Health 24: 385-402
Animals were exposed to 3.5 or 0 mg/m^3 DEP for 7 h/d, 5 d/wk for up to 17 days. Animals were sacrificed 2, 12, and 17 days after exposure. Source of DEP were two 1980, 5.7 liter Oldsmobile diesel engines burning Phillips D-2 diesel fuel.	Female F344 rats (18-20 wks old)	3.5 mg/m^3 DEP: Two inflammation mediators from alveolar cells (prostaglandin F_{2a} and leukotriene B_4) were significantly increased after 2 days, and decreased to near control levels by the 17th day. No effect on body weight, wet lung weight, or the number and distribution of cell types in the bronchoalveolar lavage fluid (BALF).	Henderson, 1988a Toxicol Letter 42: 325-332
As above.	Female $B6CF_1$ mice (13 wks old)	3.5 mg/m^3 DEP:. Two inflammation mediators from alveolar cells (prostaglandin F_{2a} and leukotriene B_4) were significantly increased after 2 days, and decreased to near control levels by the 17th day. Effects were not as severe as in rats. No effect on body or wet lung weight. Increases in the total number of cells, and the concentration of macrophages and neutrophils in the BALF.	Ibid
Animals were exposed to 6 or 0 mg/m^3 DEP, 6 mg/m^3 carbon black particulate, or 2 or 7 ppm NO_2 for 20 h/d, 7 d/wk for up to 14 days. Animals were sacrificed 1, 2, 3, 7, 11, or 14 days after exposure. Source of DEP was a 1982, 5.7 liter GM diesel engine (MMAD 0.19 µm).	Male Fischer rats	6 mg/m^3 DEP: DNA synthesis and alveolar type II cell labeling index increased significantly after two days of exposure, and returned to control levels after 7 days of exposure. There was also a significant increase in lung lipid metabolism after one day of exposure, which then returned to normal. 6 mg/m^3 carbon black: No significant changes. 7 ppm NO_2: Changes similar to those observed in animals exposed to 6 mg/m^3 of DEP.	Wright, 1986 Exper Lung Res 10: 39-55.

Table 4.3 Animal Non-Cancer Effects After Subchronic (1 to 6 months) Exposure to Diesel Exhaust.

Exposure	Animal Species	Effects/Observations	Reference
Light Duty Diesel Engine Inhalation Exposure			
Study was designed to measure spontaneous and forced activity in rats exposed to diesel exhaust particulates (DEP) after weaning. Matched pairs of animals, age 45 days, were monitored for baseline activity levels for 2 weeks. Then, one member of each pair was exposed to 0 or 6 mg/m^3 of DEP for 16 weeks. Source of DEP was 2 Nissan CN 6-33 diesel engines.	Sprague-Dawley rats	6 mg/m^3 DEP: The overall activity of the exposed animals was significantly less than that in the control animals. LOAEL = 2 mg/m^3	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4): 253-306
Animals were exposed to 0 or 6 mg/m^3 DEP for 8 h/d, 7 d/wk for 3 months. Animals were then tested for lung volume and diffusing capacity and then sacrificed. Source of DEP was 2 Nissan CN 6-33 diesel engines.	Female CR/CD-1 mice	6 mg/m^3 DEP: Slight increase in lung weights, vital capacity and CO (carbon monoxide) diffusing capacity (not significantly different from controls). LOAEL = 2 mg/m^3	Ibid
Animals were exposed to 0, 6, or 12 mg/m^3 DEP for 8 h/d, 7 d/wk for 6 months. Animals were then tested for lung volume and diffusing capacity and then sacrificed. Source of DEP was 2 Nissan CN 6-33 diesel engines.	Male Chinese hamsters	6 and 12 mg/m^3 DEP: Dose-dependent significant increase in lung weight and decrease in vital capacity and CO diffusing capacity. Microscopic evaluation revealed hyperplasia of Type II alveolar cells resulting in a thickening of alveolar lining. LOAEL = 2 mg/m^3	Pepelko and Peirano, 1983 J Am Coll Toxicol 2(4): 253-306 Vinegar *et al.*1981 Env Int 5:369-371
Animals were exposed to 1.5, 0.75, or 0 mg/m^3 DEP for 20 h/d, 5.5 d/wk for up to 6 months. Animals were sacrificed after 2 weeks, 3 months, and 6 months. Source of DEP was a 1978, 5.7 liter Oldsmobile diesel engine burning Amoco type 2D federal compliance fuel (MMAD 0.19 *u*m).	Male Hartley guinea pigs	0.75 mg/m^3 DEP: Examination of the lung revealed scattered clusters of pigmented macrophages; cellular uptake of DEP by epithelial type 1 cells, alveolar and interstitial macrophages, and eosinophils; increase in the size and number of epithelial type 2 cells. These effects were seen as early as six weeks, and increased over time. Morphometric analyses revealed significant increases in total lung tissue volume and mean tissue thickness of the air-blood barrier (primarily due to increase of interstitial and epithelial type 2 cells). Some of these effects peaked after 2 weeks, and then began to return to normal. Gas exchange function of the lung appear unimpaired. 1.5 mg/m^3 DEP: Effects on the structure of the lung were similar to, but more severe than, those observed in the lower exposure group. However, some of the morphometric changes were less severe in these animals, suggesting possible adaptive responses. LOAEL = 0.49 mg/m^3	Barnhart *et al.*, 1981 J Appl Toxicol 1(2): 88-103

Table 4.3 Animal Non-Cancer Effects After Subchronic (1 to 6 months) Exposure to Diesel Exhaust (continued).

Exposure	Animal Species	Effects/Observations	Reference
Heavy Duty Diesel Engine Inhalation Exposure			
Animals were exposed to 0 or 2 mg/m^3 DEP for 7 h/d, 5 d/wk for 1, 3 or 6 months. Animals were then inoculated intranasally with AO/PR/8/34 influenza virus. Sacrifice times were not stated in the report. Source of DEP was a Caterpillar Model 3304 diesel engine.	Female CD-1 mice	2 mg/m^3 DEP: 1 month exposure: No significant difference between exposed and controls on lung morphology and animal mortality. 3 month exposure: Significantly more mice in the exposed group exhibited lung consolidation. No difference in mortality. 6 month exposure: Hemaglutinin antibody levels were significantly less than in controls. No difference in mortality. LOAEL = 0.41 mg/m^3	Lewis, *et al.*, 1986 In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi *et al.* pp. 361-380
As above, but examined only animals exposed for one month. Animals exposed intranasally were sacrificed at 8 hours and then daily at 1 and 8 days after exposure.	Female CD-1 mice	Specific activities of two cytochrome P-450 mono-oxygeneses (7-ethoxycoumarin (7EC) and ethylmorphine demethylase (EMD)) and NADPH cytochrome c reductase (NCR) in liver microsomes were monitored. In animals affected with influenza virus pre-exposure to DEP reduced or abolished the increase in EMD and NCR but 7EC was not affected. LOAEL = 0.4 mg/m^3	Rabovsky *et al.*, 1986 Env Res 40:136-144
Animals were exposed to 2.0 or 0 mg/m^3 DEP for 7 h/d, 5 d/wk for up to 6 months. Animals were exposed for 1, 3, or 6 months, removed and inoculated intranasally with 0.05 ml of influenza virus, and then sacrificed 0 to 11 days later. Source of DEP was a Caterpillar diesel engine burning # 2 diesel fuel.	Female CD-1 mice	2.0 mg/m^3 DEP: The severity of influenza virus infection was more pronounced in mice previously exposed to DEP than in control animals. There was significantly higher numbers of animals with virus-induced lung consolidation, higher peak levels of virus multiplication, lower levels of interferon in both lung and serum, and lower levels of hemaglutinin antibody. These effects were prominent in mice exposed for either 3 or 6 months, but not in mice exposed for only 1 month. LOAEL = 0.42 mg/m^3	Hahon *et al.*, 1985 Environ Res 37: 44-60

Table 4.4 Animal Non-Cancer Effects After Chronic (7 to 27 month) Exposure to Diesel Exhaust.

Exposure	Animal Species	Effects/Observations	Reference
Light Duty Diesel Engine Inhalation Exposure			
Animals were exposed to 0, 2.44 or 6.33 mg/m^3 diesel exhaust particles (DEP) beginning at 7-9 weeks of age. Exposures were for 16 h/day, 5 d/wk for 24 months. Groups consisted of 114 to 118 animals/sex. Serial sacrifices were performed at 3, 6, 12, 18, and 23 months for lung histopathology examination.	Male and Female F344/N rats	DEP exposure resulted in increased incidence and severity of alveolar macrophage hyperplasia, alveolar epithelial hyperplasia, chronic-active inflammation, septal fibrosis, alveolar proteinosis, bronchiolar-alveolar metaplasia, and focal fibrosis with epithelial hyperplasia in animals examined 3 to 23 months afer exposure. In addition, DEP exposure resulted in increased lung weight, compared to controls. Carbon black particles were less potent in inducing these non-cancerous lesions.	Nikula *et al.*, 1995 Fundam Appl Toxicol 25: 80-94
Starting at 18 weeks, animals (50 rats in each of four study groups) were exposed to 3.5 or 0 mg/m^3 of diesel exhaust particles (as soot) for 7 h/d, 5 d/wk for 24 months. 6 weeks prior to exposure, intratracheal instillation of elastase induce mild emphysema in one half of the rats.. Animals from each group were sacrificed after 6, 12, 18, and 24 months. Source of DEP was 1980, 5.7 liter Oldsmobile diesel engines burning a standardized fuel (D-2 Diesel Control Fuel).	Male and Female F344/Crl rats	Less diesel soot accumulated in the lungs of the emphysematous rats, but none of sixty-three measured parameters indicated increased susceptibility to emphysema. Measurements included pulmonary function, cytology and enzymes in brochoalveolar lavage fluid, lung tissue collagen, immune responses in pulmonary lumph nodes, clearance of inhaled radiolabeled tracer particles, excised lung weight and volume, histopathology and terminal air space size. Accumulation of soot in lungs was accompanied by progressive focal, fibrotic and proliferative lesions. These effects were less severe in the emphysematous lungs.	Mauderly *et al.*, 1990a Am Rev Respir Dis 141: 1333-1341
Animals were exposed to 1.5 or 0 mg/m^3 DEP for 20 h/d, 5.5 d/wk for 18 months. Source of DEP was a 1978 Oldsmobile diesel engine burning Amoco typ 2D diesel fuel.	Male Harley guinea pigs	1.5 mg/m^3 DEP: significant increases in the total number of interstital cells and mononuclear cells in the alveolar regions of the lungs. No effect on the cellular population of alveolar capillaries. Adjusted LOAEL = 1 mg/m^3	Wallace *et al.*, 1987a Scanning Microscopy 1(3): 1387-1395
As above, except that animals were exposed to 6.0, 1.5, or 0 mg/m^3 DEP and were serially sacrificed after 2 weeks, 6 weeks, 10 week, or 18 months.	Male Fischer rats	Transmission electron microscopy was used to determine the effect of DEP on the alveolar region of the lungs. 1.5 and 6.0 mg/m^3 DEP: Significant increases in the volume density of the interstitium, alveolar macrophages, cellular content, and interstitial space after 10 weeks and 18 months of exposure. Significant increases in the total number of interstitial cells, fibroblasts, and mononuclear cells in these two exposed groups when compared to controls. Adjusted LOAEL = 1 mg/m^3	

Table 4.4 Animal Non-Cancer Effects After Chronic (7 to 27 month) Exposure to Diesel Exhaust (continued).

Exposure	Animal Species	Effects/Observations	Reference
Starting at 13 months, animals were exposed to 6.34 or 0 mg/m^3 DEP for 8 h/d, 7 d/wk for 61 weeks, and then exposed to 11.7 or 0 mg/m^3 DEP for 8 h/d, 7 d/wk for 62 weeks, for a total of 27 months of exposure. A subgroup of animals was sacrificed after 27 months. Remaining animals were placed in clean air chambers for 6 months and sacrificed 33 months after the start of the experiment. Source of DEP was a Nissan CN6-33 engine burning number 2 diesel fuel.	Male cats	6 and 12 mg/m^3 DEP: there was a decrease in lung weight in exposed animals sacrificed after 27 months, and an increase in lung weight and decrease in lung volume in exposed animals sacrificed after 33 months. The bronchiolar epithelium of exposed animals showed micronodular proliferations, composed primarily of nonciliated cells, numerous ciliated cells, increased cuboidal cell-lined outpockets of bronchiolar walls, and aggregations of alveolar macrophages within alveoli of brochioloes. All of these effects showed some recovery in animals allowed six months exposure to clean air. However, peribronchiolar connective tissue fibrosis and related lung collagen levels continued to increase in these animals, although this group showed significantly fewer fibroblasts than animals without this recovery peroid. This connective tissue was filled with particle laden macrophages in both exposed groups. Lymphocytes were increaed in the group with no recovery period, while those with a recovery period had more eosinophils and anorphous matrix. Eosinophils were reduced in both groups when compared to controls.	Hyde *et al.*, 1985 Laboratory Investigation 52(2): 195-206
Animals were exposed to 0 or 6 mg/m^3 DEP for 8 h/d, 7 d/wk for 1 year, then continued exposure for an additional 15 months at 12 mg/m^3. Source of DEP was 2 Nissan CN 6-33 diesel engines.	Male cats	6 mg/m^3 DEP: At one year, there were no observable changes in lung function. 12 mg/m^3 DEP : At two years there was a well -defined response including decreased lung volume, peak expiratory flow rate, diffusing capacity and an increase in nitrogen in expired air at 25% of vital capacity. Microscopic evaluation of bronchioles revealed hyperplasia of epithelial cells, increased numbers of intraluminal inflammatory cells, presence of ciliated cells in the epithelium, and an increase in the number of bronchoalveolar communications. Simple cuboidal nonciliated broncholar cells were replaced by pseudo-stratified columnar epithelium. Blood and serum biochemistry evalustions did not show any consistent effects.	Pepelko and Peirano, 1983 J Amer Coll toxicol 2(4)253-306

Table 4.4 Animal Non-Cancer Effects After Chronic (7 to 27 month) Exposure to Diesel Exhaust (continued).

Exposure	Animal Species	Effects/Observations	Reference
Animals were exposed to 1.5, 0.25, or 0 mg/m^3 DEP for 20 h/d, 5.5 d/wk for up to 1 year. Animals were sacrificed after 1.5, 3, 6 or 12 months. Source of DEP was a GM 1978 production model diesel engine burning AMOCO type 2D diesel fuel.	Hartley guinea pigs	0.25 mg/m^3 DEP: Animals exhibited a significant increase in pulmonary prostaglandin dehyrogenase (PGDH) activity after 1.5 months, which decreased thereafter. Levels did not differ significantly from controls at 1 year. 1.5 mg/m^3 DEP: Animals exhibited a significant decrease in PGDH activity at 1.5, 3 and 6 month timepoints. However, levels did not differ from controls at 1 year. Adjusted LOAEL = 0.16 mg/m^3	Chaudhari *et al.*, 1981 J Appl Toxicol 1(2): 132-134 (methods described i Schreck, 1981)
Animals were exposed to 1.5, 0.75, or 0 mg/m^3 DEP for 20 h/d, 5.5 d/wk for up to 9 months and sacrificed after 1, 3, 6 or 9 months. Source of DEP was a GM 1978 production model diesel engine burning AMOCO type 2D diesel fuel.	Male Fischer-344 rats	1.5 mg/m^3 DEP: Animals exhibited a slight but significant increase in liver aryl hydrocarbon hydroxlase (AHH) activity, and a slight decrease in liver cytochrome P-450 after 1 month. These changes were not observed in animals exposed to 0.75 mg/m^3 DEP. Adjust LOAEL = 1 mg/m^3	Chen and Vostal, 1981 J Appl Toxicol 127-131 (methods described in Schreck, 1981)
Animals were exposed to 0, 0.25, 0.75, or 1.5 mg/m DEP for 20 h/d, 5.5 d/wk for up to 1 year. Animals were sacrificed at various timepoints during exposure to determine effect of exposure on hepatic and pulmonary microsomal activity.	Male Fischer rats	0.25, 0.75, and 1.5 mg/m^3 DEP: Pulmonary microsome metabolic abilities, as measured by generation of BaP polar metabolites, was impaired at 1 year after exposure. No alteration in liver microsome oxidation ability, levels of cytochrom P-450, P-448, or NADPH-dependent cytochrome and reductase was observed in DEP-exposed animals. Adjusted LOAEL = 0.16 mg/m^3	Navarro, *et al.*, 1981 J Appl Toxicol 1(2) 124-126
Animals were exposed to 0, or 2 mg/m^3 diesel exhaust alone or in combination with coal dust 7 h/day, 5 d/week, for up to 24 months	Male cynomolgus monkeys	Results of exposed monkeys showed a significant increase in mild pulmonary acute and chronic inflammatory responses. Exposed animals also exhibited reduced pulmonary flow rate (obstructive disease), but not reduced lung volume (restrictive disease). The obstructive responses began to occur significantly after 12 months of DEP exposure. Adjusted LOAEL = 0.4 mg/m^3	Lewis *et al.* 1986 Dev Toxicol Environ Sci 13: 361-380.

Table 4.4 Animal Non-Cancer Effects After Chronic (7 to 27 month) Exposure to Diesel Exhaust (continued).

Exposure	Animal Species	Effects/Observations	Reference
Heavy Duty Diesel Engine Inhalation Exposure			
Starting at three weeks, animals were exposed to 2.0 or 0 mg/m^3 DEP for 7 h/d, 5 d/wk for 24 months (MMAD 0.23 μm). Source of DEP was a four cylinder Caterpillar diesel engine burning number 2 diesel fuel.	Male and female Fischer 344 rats, 7-8 weeks old	2.0 mg/m^3 DEP: Focal accumulation of particle-laden alveolar macrophages associated with mild type II cell hyperplasia of the alveolar lining cells. Pigmented macrophages were observed in the interstitium, but there was no fibrosis. There was also an insignificant increase in the thickness of the pulmonary artery walls. No effect on heart and body weight. Adjusted LOAEL = 0.4 mg/m^3	Vallyathan *et al.*, 1986 J Toxicol and Enviro Health 19: 33-41
Animals were exposed to 2.0 or 0 mg/m^3 DEP for 7 h/d, 5 d/wk for 2 years (MMAD 0.23-0.36 μm). Exposure were conducted during the dark period of the light/dark cycle. Animals were sacrificed after 24 months. Source of DEP was a Caterpillar 3304 diesel engine burning number 2 diesel fuel.	Male Fischer 344 rats	2.0 mg/m^3: Exposed animals exhibited decreased alveolar macrophage (AM) volume, although their number remained the same; there was no effect on AM membrane integrity, oxygen consumption, or protein synthesis; the only effect on lysosomal enzymes was an increase in beta-N-acetylglucosaminidase activity. Exposure to DEP depressed chemiluminescence generated by AM at rest and decreased AM phagocytic activity. Viability of AM was not decreased. Adjusted LOAEL = 0.4 mg/m^3	Castranova *et al.*, 1985 Environ Res 36 405-419

Table 4.5 Summary of the Reproductive and Developmental Effects of Diesel Exhaust Exposure.

Exposure	Species	Observations	Reference
Male Reproductive Studies			
Sperm Abnormalities Study: Male mice were exposed to clean air or 6 mg/m^3 DEP 8 h/d, 7 d/wk for 31 or 39 weeks. These exposure periods were chosen since they represent approx. 6 and 8 complete spermatogenic cycles. Animals were sacrificed at the end of exposure and sperm shapes were analyzed from preparations of excised cauda epidiymides.	Male Strain A mice	No difference in the proportion of sperm abnormalities between control and exposure group.	Pereira *et al.*, 1981 Environ Int 5:439-443
Sperm Abnormalities Study: Male hamsters were exposed to clean air or 6 mg/m^3 DEP for 8 h/d for 6 months. Animals were sacrificed at the end of the exposure period. Percentage of abnormal sperm was recorded.	Male Chinese hamsters	A 2.67 fold-increase in sperm abnormalities was observed in DEP exposed animals	Pereira *et al.*, 1981 Environ Int 5:459-60
Point mutation study: Groups of male mice were exposed to 6 mg/m^3 DEP, 8 h/d, 7 d/wk for 5 or 10 weeks. Males were then mated to 4 multiple recessive "T" stock females. These females were homozygous recessive for seven phenotypic traits. Males were supplied with 4 new females once per week for 4-5 weeks. Diesel particles were generated by 6 cylinder Nissan engine run on Federal Short Cycle. BaP concentration 16 ug/g diesel particle.	(101 x C_3H)F_1 or (C_3H x 101)F_1 hybrid male and 'T' Stock female mice	No evidence of mutations	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4)253-306
Induction of dominant lethal mutations: Male mice were exposed to clean air or 6 mg/m^3 DEP, 8 h/d, 7 d/wk for 7.5 weeks. Each exposure group was randomized into 4 subgroups. Each subgroup was mated with one of 4 different stocks of females.. Each male was caged with 2 females. Mated females were replaced with new ones. The mating period was for 7 consecutive days. Mated females were killed on day 12-15 of gestation and evaluated for number of implants, dead implants and live embryos.	"T" stock male and (C_3H x 101)F_1; (SEC x C57BL)F_1, (C_3H x C57BL)F_1, or "T" stock female mice	No differences were observed in the number of females pregnant, the number of implants/female, the number living embryos/female, or the percent dead implants between controls and DEP exposed groups. No evidence of dominant lethal mutations in male germ cells.	ibid

Table 4.5 Summary of the Reproductive and Developmental Effects of Diesel Exhaust Exposure (continued).

Exposure	Species	Observations	Reference
Male Reproductive Studies (continued)			
Heritable translocation mutations: Male mice were caged with 3 (SEC x C57BL)F_1 females prior to exposure to produce offspring for estimating control frequency of translocations. After pre-exposure mating, males were exposed to clean air or 6 mg/m^3 DEP, 8 h/d, 7d/wk for 4.5 weeks. Exposed males were then caged with 2 (SEC x C57BL)F_1 females for 1 week. All male progeny were tested for sterility and partial sterility.	Male stock "T" and (SEC x C57BL)F_1 female mice	No difference between control and exposure frequencies. No evidence of translocation mutations.	ibid
Spermatogonial Survival: Male mice were exposed to clean air or 6 mg/m^3 DEP 8 h/d, 7d/wk for 5 or 10 weeks. Animals were killed shortly after exposure was terminated and testes were evaluated histologically. Eight spermatogonial stages and preleptotene spermatocytes were enumerated.	Hybrid male JH and H strains	No effects of diesel exhaust exposure were observed.	ibid
Diesel particulate suspended in corn oil was administered daily by intraperitoneal injection (i.p.) for 5 days to male mice at doses of 50, 100 and 200 mg/kg. Percentage of teratospermia, testicular weights and sperm counts were scored.	(C57B1/6 x C3H)F_1 male mice (12 weeks of age)	Statistically significant increases in sperm abnormalities were noted in treated animals. Decrease in sperm number was observed in the 200 mg/kg group but testicular weight was not affected.	Quinto and DeMarinis, 1984 Mutat Res 130:242
Induction of dominant lethal mutations: Rats were exposed to 0 or 2 mg/m^3 DEP 7 h/d, 5 d/wk for 6 months. Following exposure males were cohabited with 3 females for 7 consecutive days after which 2 additional females were added. Source of diesel exhaust was a 425 in^3, 7 liter Caterpillar engine (Model 3304), MMAD 0.23 - 0.36 *u*m.	Fischer 344 rats, weanling males	The number of uterine live implants, dead implants and preimplantation losses were equivalent between the treatment groups indicating no dominant lethal effects were observed.	Lewis et al, 1986 In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi *et al.*, pp 361-380.
Semen evaluations: Cynomologus male monkeys were exposed by inhalation for 7 h/d, 5d/wk for 24 months to 0 or 2 mg/m^3 DEP. Sperm analyses were performed after recovering a sample of spermatozoa from the left cauda epidiymides of 59 monkeys. Sperm counts, motility, velocity and sperm head morphology were evaluated.	Cynomolgus male monkeys (*Macaca fascicularis*)	No statistically significant distributional difference were observed between the treatment groups for any of the spermatogenic indices evaluated.	ibid

Table 4.5 Summary of the Reproductive and Developmental Effects of Diesel Exhaust Exposure (continued).

Exposure	Species	Observations	Reference
Male Reproductive Studies (continued)			
Dominant lethal mutation study: Animals were exposed to tank-generated diesel exhaust/smoke (2,340 mg/m^3), exhaust only (6 mg/m^3), or tank noise only 15 or 60 min/d, 5 d/wk for 10 weeks. Each male was placed with 2 females for 5 days. Females were replaced with 2 fresh females for another 5 days. Pregnancy rate and fetal viability were assessed on day 11 of gestation. Source of diesel exhaust was a M60A1 tank (MMAD 0.29 µm)	Sprague-Dawley male rats	No exposure related effects were observed.	Callahan *et al.*, 1986 Chemical Research and Development Center Report
Female Reproductive Studies			
Induction of genetic effects and oocyte killing study. Female mice were exposed to clean air or 6 mg/m^3 DEP 8 h/d, 7d/wk for 8 weeks. Each female was caged with 1 (C_3H x 101)F_1 male. Reproductive success was determined by the size of the litter.	Female (SEC x C57BL)F_1 and male (C_3H x 101)F_1 mice	Similar pregnancy rates (85% vs 86%) and average litter sizes (11.4 vs 11.4) were observed between groups. No evidence of chromosomal or cytotoxic effects in oocytes.	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4)253-306
Dominant lethal mutation induction study. Female mice were exposed to clean air or 6 mg/m^3 DEP for 8 h/d, 7d/wk for 7 weeks. Females were caged with (101 x C_3H)F_1 males. At approximately 14 days of gestation females were killed and evaluated for successful mating, corpora lutea (i.e. estimation of the number of ova ovulated), number of live embryos, preimplantation loss, postimplantation loss, and mean time between caging and copulation (i.e. mating interval).	Female (101 x C_3H)F_1 and male (101 x C_3H)F_1 mice	No significant differences were found between groups for the parameters: successful mating (% of matings resulting in pregnancy); number of live embryos; preimplantation loss and postimplantation loss. Therefore, no evidence of dominant lethal effects was observed. The diesel exposed females, however, did have signifcantly fewer corpora lutea per female than did controls, 9.37 vs 10.85 ($p<0.0001$). Diesel exposed females also exhibited longer mating intervals than controls, 4.42 days vs 2.94 days ($p<0.016$).	ibid
Teratogenicity Studies			
Animals were exposed to clean air or 6 mg/m^3 DEP 8 h/d during day 5 through 16 of gestation. Animals were killed 1 day before the predicted parturition date and fetuses were examined for gross internal and skeletal abnormalities. In addition to abnormalities, the following parameters were also recorded: total number of fetuses, number of implantation sites, number of corpora lutea, distribution of fetuses in the uterine horns, gross pathology of the dam, individual fetal weight, litter weights and sex ratio of the offspring.	Sprague Dawley rats	None of the reproductive parameters assessed differed significantly between control and DEP exposure groups. No conclusive effects were noted for either fetal visceral or skeletal abnormalities.	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4)253-306
As above	New Zealand White Rabbits	None of the reproductive parameters assessed differed significantly between control and diesel exhaust exposure groups. No conclusive effects were noted for either fetal visceral or skeletal abnormalities.	ibid

Table 4.5 Summary of the Reproductive and Developmental Effects of Diesel Exhaust Exposure (continued).

Exposure	Species	Observations	Reference
Female Reproductive Studies (continued)			
Pregnant animals were exposed to tank-generated diesel exhaust/smoke (2,340 mg/m^3), diesel exhaust only (6 mg/m^3) or tank noise for 60 min/d, from day 6 through 15 of gestation. Pregnant animals were killed on day 20 of gestations and the fetuses were removed and evaluated for viability, sex, weight, visceral anomalies and skeletal anomalies. Source of diesel exhaust was a M60A1 tank (MMAD 0.29 µm)	Sprague-Dawley rats	Sites of retarded ossification were found to be significantly higher in the vertebral columns, ribs and sternums of pups exposed to emissions than those exposed to tank noise only suggesting fetal toxicity and/or delayed development.	Callahan *et al.*, 1986 Chemical Research and Development Center Report
Generational Studies			
Animals were exposed to clean air or 12 mg/m^3 DEP for 8 h/d, 7 d/wk. F_0 and F_1 generation males and females were exposed for 100 days prior to breeding. At start of breeding 1 male was housed with 1 female. 100 male-female pairs were randomly assigned to 4 exposure groups (see p. 292). Pairing was continued until evidence of a copulatory plug or until 15 consecutive days had elapsed. Breeding males were killed 10 days after evidence of mating. Breeding females were removed and killed when litters reached weaning age. Remaining F_1 mice not designated for breeding were kept in their respective exposure environments for 176 to 196 days and then killed. F_2 offspring were kept in their respective exposure environments until 42 days of age and then killed.	CD-1 mice	Body weight: generally no difference between exposure groups in the F_0 and F_1 generations. Some depression in the F_2 generation. Fertility data: no differences between exposure groups were detected for any of the parameters. Parameters measured: gestation length, % fertile, mean litter size at birth, and % pup survival at day 1, 4, and 21. Only consistent change was an increase in the lung weight of the diesel exposed groups which was accompanied by a gross pathological diagnosis of anthracosis.	Pepelko and Peirano, 1983 J Amer Coll Toxicol 2(4)253-306
Male animals were exposed to tank-generated diesel exhaust/smoke (2,340 mg/m^3), diesel exhaust only (6 mg/m^3) or tank noise for 15 or 60 min/d, 5 d/wk for 10 weeks. Female animals were exposed to the same environments for the last 3 weeks of male exposure period. In the week following exposure 2 females were caged with each male. The females were then returned to their daily exposure environments through weaning of their neonates (the neonates were not exposed). Source of diesel exhaust was a M60A1 tank (MMAD 0.29 *u*m)	Sprague-Dawley rats	No differences between control and exposed groups were observed regarding mating, the period of gestation, deliver and care of neonates. Temporary decrease of body weight was noted in neonates of treated animals, but the effect was no longer apparent at 21 days of age.	Callahan et al, 1986 Chemical Research and Development Center Report

Table 4.5 Summary of the Reproductive and Developmental Effects of Diesel Exhaust Exposure (continued).

Exposure	Species	Observations	Reference
Developmental Studies			
Neurobehavioral: Two groups of animals were exposed to clean air or 6 mg/m^3 DEP. Group 1: exposed from day 1 to 17 of age for 20 h/day, 7 d/wk. Group 2: exposed from day 1 to 21, 28 or 42 of age for 8 h/d/m 7 d/wk. Spontaneous activity was evaluated in Groups 1 and 2 starting at 7 weeks of age and continued for 16 weeks. Operant conditioning testing was evaluated in Group 1 only starting at 15 months of age and continued for 42 days at 5 day intervals.	Sprague-Dawley rat pups	Spontaneous Activity: Group 1: Overall diesel exhaust exposed animals were significantly less active than controls. Group 2: Activity in diesel exhaust exposed animals was depressed, but depression was less dramatic than in Group 1. Significant differences were observed at weeks 5 through 13 of testing. Operant Conditioning: Controls showed a sharp rise in learning at day 10 of testing and all learned the task shortly thereafter. In diesel exhaust exposed animals by day 25 only 1 out of 10 animals had learned task. Eventually all diesel exhaust exposed animals learned the task within the test period (42 days).	Ibid, and Laurie *et al.*, 1981 Environ Int 5:357-361
Neurophysiological: Animals were exposed from birth to day 7, 14, 21, or 28 of age to 0 or 6 mg/m^3 DEP 8 h/d, 7d/wk. Development of the nervous system was measured by somatosensory and visual evoked potential (SEPs and VEPs, respectively).	Sprague-Dawley rat pups	Significant difference in the latency of the SEPs and VEPs occurred only in the 1 to 14 day exposure group. Note: The most rapid rate of growth occurs at about 14 days of age.	Ibid, and Laurie and Boyes, 1981 Environ Int 5:363-368
Lung: Animals were exposed to 0 or 3.5 mg/m^3 DEP 7 h/d, 5 d/wk from conception till 6 months of age. Body weight, respiratory function, pulmonary immune response, lung clearance of radiolabeled particles, airway fluid enzymes, lung tissue collagen, proteinase, lung burden of diesel particles, lung morphometry and histopathology were measured. Source of diesel exhaust was a 1980 Model 5.7 liter Oldsmobile engine.	Fischer 344 male rats	Diesel exhaust exposure altered the airway fluid constituents (acid phosphatase, LDH, and glutathione reductase) as well as lung enzymes. Lung tissue collagen was significantly increased whereas proteinase was significantly decreased. Effects observed were much less severe than in adults exposed to the same diesel exhaust levels. The authors stated that in general normal lung development was not affected by diesel exhaust exposure. Exposure time frame included the major phases of rat lung development: *in utero*, neonatal, and rapid growth during maturation.	Mauderly et al, 1987 Health Effects Institute Report

Table 4.6 Rat Lung Hyperplasia Incidence Following Diesel Exhaust Exposure (Ishinishi *et al.*, 1988).

DIESEL TYPE	DIESEL CONCENTRATION				
Heavy duty	0 mg/m^3	0.46 mg/m^3	0.96 mg/m^3	1.84 mg/m^3	3.72 mg/m^3
Male	0/64	2/64	4/64	4/64	8/64
Female	1/59	1/59	3/61	10/59	17/60
Light duty	0 mg/m^3	0.11 mg/m^3	0.41 mg/m^3	1.18 mg/m^3	2.32 mg/m^3
Male	4/64	3/64	2/64	4/64	38/64
Female	0/59	1/59	4/61	8/59	49/60

Table 4.7 Summary of Non-Cancer Guidance Values and Benchmark Concentrations from Experimental Diesel Exhaust Studies (from Table 10.5, WHO, 1994).

Approach	Guidance Value or Benchmark Concentration (μg/m^3)
NOAEL using rat-human dosimetric conversion	5.6
NOAEL without rat-human dosimetric conversion	2.3
Benchmark concentration using rat-human dosimetric conversion	
chronic alveolar inflammation	2
impaired lung clearance	3
hyperplastic lesions	1
Benchmark concentration without rat-human dosimetric conversion	
chronic alveolar inflammation	0.9
impaired lung clearance	1.3
hyperplastic lesions	6.3

Table 4.8 Benchmark Concentrations Using Probit and Weibull Models (TOX-RISK V. 3.5) on Female Rat Lung Hyperplasia (Ishinishi *et al.*, 1988).

% Additional risk	Maximum Likelihood Estimate (mg/m^3)	95% Lower Bound (mg/m^3)	Equivalent Reference Concentration ($\mu g/m^3$)
Probit model			
1	0.46	0.17	3
5	1.02	0.59	11
10	1.58	1.10	21
Weibull model			
1	0.32	0.11	2
5	1.00	0.54	10
10	1.64	1.12	21

5.0 GENOTOXICITY

Genotoxicity tests can provide insight into mechanisms of carcinogenicity, reproductive toxicity and other genetically influenced processes caused by DNA-reactive agents. If DNA adducts are not repaired, then, during cell replication a point mutation or chromosomal alteration can occur. Altered oncogene or lost tumor-suppressor-gene function may be in part responsible for the carcinogenicity of DNA-reactive agents (Tong *et al*., 1989; Marshall *et al*., 1991; Solomon *et al*., 1991; Weinberg *et al*., 1991). Short-term genotoxicity tests can be completed quickly and inexpensively and are useful in determining the bio-active properties of complex mixtures such as diesel exhaust.

This section surveys diesel exhaust-, diesel exhaust particulate-, and diesel exhaust extract-induced genotoxicity in bacteria, yeast, Drosophila, rodents, non-human primates and humans. Further review may be found in IARC (1989), HEI (1995) and WHO (1996). Much of the information regarding genotoxicity has been obtained using diesel exhaust particles or extracts of diesel exhaust particles. Diesel exhaust particles or their extracts are mutagenic in bacteria (*Salmonella typhimurium* and *E. coli*) and in several mammalian cell systems (CHO, V79, BALB/c3T3, L5718Y mouse lymphoma, human lymphoblasts). Diesel exhaust particles or their extracts induce chromosome aberrations, aneuploidy, and sister chromatid exchange in rodent and human cells in culture. Diesel exhaust particles and their extracts are also capable of inducing cell transformation. Diesel exhaust particles or their extracts can also produce superoxide and peroxide radicals and inhibit the antioxidant enzymes responsible for radical scavenging. Both diesel exhaust particle extracts and the semivolatile phase of diesel exhaust have dioxin receptor binding affinity. Exposure to diesel exhaust particulate matter can cause unscheduled DNA synthesis *in vitro* in mammalian cells. DNA adducts have been isolated from calf thymus DNA *in vitro* and mouse lung DNA following intratracheal instillation.

Some information regarding genotoxicity also has been obtained directly from diesel exhaust exposures. Whole diesel exhaust has been demonstrated to induce gene mutations in two strains of Salmonella. Inhalation exposure to diesel exhaust results in DNA adduct formation in rodents and monkeys. Increased levels of human peripheral blood cell DNA adducts are associated with occupational exposure to diesel exhaust. The genotoxic effects of diesel exhaust may be involved in the initiation of pulmonary carcinogenesis in humans.

Diesel exhaust clearly contains genotoxic substances. Furthermore, diesel exhaust particles and diesel exhaust extracts have been established to be genotoxic. The bioavailability of these genotoxins has been questioned. Several lines of evidence suggest bioavailability. First, the *in vitro* genotoxic activity of diesel exhaust particulates dispersed in pulmonary surfactant exhibited similar activity to particulates extracted with dichloromethane. Second, inhalation exposure of rats and monkeys to diesel exhaust results in DNA adduct formation and *in vitro* exposure of rat tissues to diesel exhaust induces unscheduled DNA synthesis. Third, DNA adducts have been associated with occupational exposure to diesel exhaust. Fourth, urinary metabolites of PAHs have been found following exposure of rats to diesel exhaust. Preliminary evidence indicates the same may be true for humans. Consequently, it appears that organic chemicals adsorbed onto the particles, particularly the genotoxic components, are likely to be bioavailable in humans.

5.1 TESTS ASSESSING GENE MUTATION

Diesel exhaust particles or their extracts are mutagenic in bacteria (*Salmonella typhimurium* and *E. coli*) and in several mammalian cell systems (CHO, V79, BALB/c3T3, L5718Y mouse lymphoma, human lymphoblasts).

5.1.1 BACTERIAL ASSAYS

Organic extracts from diesel engine exhaust were first found to be mutagenic in the Salmonella reverse mutation assay in the late 1970's (Huisingh *et al*., 1979). Salmonella typhimurium strains TA98 and TA100 proved to be the most sensitive. Strain TA1535 gave weakly positive or negative results. Whole diesel exhaust (Courtois *et al*., 1993) and the semivolatile phase of diesel exhaust (Westerholm *et al*., 1991; Sera *et al*., 1994) have also been demonstrated to be mutagenic in Salmonella. Some investigators have observed that diesel particulate extracts do not require an exogenous metabolic activation system to demonstrate mutagenic activity and that addition of such a system can reduce the number of revertants generated (McClellan, 1987; Crebelli *et al*., 1991). Other studies have shown mutagenic effects with or without metabolic activation (IARC, 1989). The general consensus is that a large portion of diesel particulate mutagenicity is direct-acting, and endogenous bacterial metabolism is involved in the bioconversion of select pro-mutagenic compounds to their mutagenic form (Rosenkranz, 1982).

Characterization studies with the Salmonella reverse-mutation assay determined that a substantial amount of the direct-acting mutagenicity of diesel engine exhaust is found in the particulate phase. When particulate extracts were fractionated into nonpolar, moderately polar and polar fractions, more than 50% of the direct-acting activity occurred in the moderately polar fraction (Schuetzle, 1983). Chemical fractionation based on acid/base properties showed that most of the mutagenic activity was in the neutral and acidic fractions. Further studies implicated certain nitroarenes (1-nitropyrene; 3- and 8-nitrofluoranthene; 1,3-, 1,6- and 1,8-dinitropyrene; 3-nitrobenzanthrone) as important mutagenic species in the more mutagenic fractions (Salmeen *et al*., 1984; Enya *et al*., 1997). A decrease in mutagenicity in nitro-reductase deficient bacterial strains also provided evidence for the role of nitro-PAHs as important mutagens in diesel engine exhaust (Claxton, 1983; Crebelli *et al*., 1991) while generating concern that bacterial mutagenic assays may overestimate the mutagenic activity of diesel exhaust (Lewtas, 1986). Likewise, the observation that nitro-PAHs could be formed during sampling triggered a debate over the possible role of these compounds as mutagenic artifacts. However, the work of Schuetzle *et al*. (1983) showed that conversion of PAHs to nitro-PAHs during dilution tube sampling of particulates is of minor concern and that, for example, most 1-nitropyrene measured in diesel exhaust particulate extracts is formed in the engine and/or tailpipe.

Additionally, Sera *et al*. (1994) detected the presence of the mutagenic nitro-PAHs 1- and 3-nitro-6-azabenzo[a]pyrene and 1- and 3- nitro-6-azabenzo-[a]pyrene-N-oxide in the semivolatile phase of diesel exhaust. The parent compounds of those nitro-PAH (azabenzo[a]pyrene and azabenzo[a]pyrene-N-oxide) were not found in either the particulate or semivolatile phase, suggesting that the nitro-PAHs were not formed artifactually during sampling.

Ball *et al*. (1990) tested the mutagenicity of eight fractions of extract with strains TA-98 and TA-100. The unfractionated extract had the previously determined characteristic of mutagenic activity being greater without S9 liver extract than with S9. The fraction containing the classical polycyclic aromatic hydrocarbons (PAH) had a mutagenicity that was slightly greater with S9 than without, consistent with the indirect mutagenicity of the PAHs. The greatest mutagenic activity was in the fractions that contained the most potent direct-acting nitroarenes, the mono- and di-nitropyrenes/fluoranthenes. The fraction in between the one with classical PAHs and the one with potent nitropyrenes showed strong indirect mutagenic activity. The investigators tentatively identified 9-nitroanthracene and other nitro-PAHs in this fraction.

Crebelli *et al*. (1991) exposed strains TA98 and TA98/1,8DNP$_6$ (O-acetyl-transferase-deficient) to acidic, neutral and basic fractions of crude organic extracts of diesel engine exhaust. Metabolic activation decreased mutagenic activity in the acidic and neutral fractions, but increased activity in the basic fraction in both strains. The authors indicated that this suggested the presence of nitrogen-containing, indirect mutagens (e.g. azaarenes) in the basic fraction.

Westerholm *et al*. (1991) characterized the composition and mutagenicity of particulate-, semivolatile- and gas phase-associated compounds in diluted heavy-duty diesel engine exhaust. The PAH content of the semivolatile phase was approximately 3-fold greater than that of the particulate phase. Mutagenicity of the diesel exhaust phases was characterized using Salmonella strains TA98 and TA100 (with and without rat liver S9). The semivolatile phase contributed approximately 10, 20 and 37% to the total mutagenicity in strains TA98 (-S9), TA100 (± S9) and TA98 (+S9), respectively.

Diesel exhaust extracts have also been demonstrated to be mutagenic in Escherichia coli WP2 (Lewtas, 1986), WP2uvrA (Crebelli *et al*., 1991) and K12 (Lewtas, 1986). In the WP2 strains, results were positive in the absence of metabolic activation. The results in the WP2uvrA strain suggest the presence of other potent direct-acting mutagens in diesel engine exhaust in addition to nitropyrenes, which are almost completely nonmutagenic in this strain (Crebelli *et al*., 1991). With *E. coli* K12, mutagenic activity was observed only after addition of a metabolic activating system.

5.1.2 FACTORS AFFECTING MUTAGENICITY IN BACTERIAL ASSAYS

5.1.2.1 FUEL

Huisingh *et al*. (1978) found that diesel fuel produced negative results when tested directly in Salmonella typhimurium, supporting the hypothesis that fuels contain the precursor material for mutagens later found in the exhaust emissions. Research comparing the mutagenic activity of combustion organics emitted from two cars operated with each of five different fuels showed that the fuel with the lowest cetane value and the highest BaP content and highest nitrogen content generated the most mutagenicity (Huisingh *et al*., 1978). Rasmussen (1990) studied the effect of varying fuel properties (aromaticity, sulfur content and boiling point) using each of nine different fuels in a single heavy duty engine. Increasing mutagenicity of the soluble organic fraction of the particulate, as determined with the Salmonella assay, was correlated with increasing fuel

aromatic content but not with sulfur content. Crebelli *et al*. (1995) also noted a positive correlation between diesel fuel aromaticity and mutagenic activity in Salmonella. Sjögren *et al*. (1996) studied the relationships between fuel characteristics and the biological effects (Salmonella mutagenicity and A*h* receptor affinity) of diesel exhaust particulate extracts using partial least squares regression. Cetane number and upper distillation curve points were found to be negatively correlated with mutagenic activity, while density, flash point, PAH content and sulfur content were found to be positively correlated with mutagenic activity. In an earlier review, Lewtas (1982a) pointed out that fuel properties have varying effects on emissions. Studies suggest that fuel with relatively high aromatic and nitrogen content can cause an increase of mutagenic emissions in certain vehicles.

5.1.2.2 VEHICLE AND ENGINE TYPE

Differences in mutagenicity between exhaust samples from light-duty diesel passenger vehicles of the same make, model, and configuration were larger than for multiple samples taken from one vehicle. A nearly ten-fold difference in revertants per microgram of organic material was observed between two automobiles (Claxton and Kohan, 1981). Results of studies comparing differences in mutagenic activity between light- and heavy-duty diesel powered vehicles revealed that the number of revertants per microgram was usually much lower for the heavy-duty samples but was similar on a revertant per mile basis due to the heavy-duty vehicles' increased emissions (Zweidinger, 1982).

5.1.2.3 OPERATIONAL CHARACTERISTICS

Studies of the effect of driving cycles on mutagenicity demonstrated the highest mutagen production in association with acceleration and high speed cruises (Bechtold *et al*., 1984). Similarly it was found that stops and starts resulted in higher mutagenic activity (Claxton, 1983). However, investigations of the mutagenicity of extracts collected under simulated highway, urban and congested urban driving cycles did not show significant differences reported as revertants/µg or revertants/mile despite large differences in particle emission rates and extractable fractions of the particles (Clark *et al*., 1982). Extracts of particles from lower mileage cars (less than 4000 miles) produced more mutagenic activity for any driving cycle tested (Claxton, 1983). Investigations of the effect of cold start temperature showed that decreasing temperature results in increased mutagenicity (ibid.).

5.1.2.4 SAMPLING

Salmeen *et al*. (1985) and Pierson *et al*. (1983) found no differences between the mutagenicity of particulate samples collected on filters in the laboratory using a dynamometer and the mutagenicity of particulate samples collected on filters located in a traffic tunnel. Also, they found the composition obtained in the two situations to be similar.

5.1.2.5 AMBIENT CONDITIONS

In a study designed to determine the effect of environmental conditions on the mutagenicity of diesel exhaust, Claxton and Barnes (1981) injected diesel exhaust directly from the engine into a smog chamber, simulating conditions in outdoor air. The investigators determined the mutagenicity of DMSO-extracted samples collected from the chamber, using four strains of Salmonella typhimurium. The effect of standing in the chamber in the dark or being subjected to irradiation for six hours did not have any substantial, consistent effect on mutagenicity of the exhaust. Introduction of ozone into the chamber reduced mutagenic activity of the sample below that from the simple exhaust atmosphere in the chamber.

5.1.2.6 BIOAVAILABILITY UNDER PHYSIOLOGICAL CONDITIONS

The detection of substantial mutagenicity obtained by using a strong organic solvent such as dichloromethane to extract the particulate matter from samples of diesel exhaust suggested the need to investigate the extent to which mutagens bound to the diesel exhaust particles could be made bioavailable under physiological conditions *in vivo*.

Siak *et al*. (1981) found that simulated body fluids (saline, bovine serum albumin to simulate proteinaceous material in body fluids and dipalmitoyl lecithin to simulate lung surfactant, and fetal calf serum to represent body fluids with complex properties) were incapable of significantly removing mutagens from diesel particles. Only fetal calf serum displayed some ability to extract the mutagenic activity (approximately 50% of that made available by dimethyl sulfoxide extraction) from the particles. Likewise, Brooks *et al*. (1981) found little mutagenic activity extracted by incubation of diesel exhaust particles with dog serum, dog lung lavage fluid, saline, albumin, or dipalmitoyl lecithin. The authors noted that the lowered mutagenic activity associated with biological media extraction compared to extraction with organic solvents could be due either to 1) “a lack of removal of mutagens from the particles”, or 2) “an inactivation of removed mutagens by protein binding or other processes”. King *et al*. (1981) found a small increase in mutagenicity of diesel soot when extracted with human serum but not when extracted with lung lavage fluid. That study and Clark and Vigil (1980) also found that the mutagenicity of dichloromethane extracts of the soot decreased with the addition of serum components and lung cytosol. The results are consistent with protein binding to the extracted mutagens, thus reducing their mutagenicity. Hence, complex body fluids may release the mutagens from the soot and also permit binding by proteins. King *et al*. (1981) also found that excitation and emission fluorescence spectroscopy data indicated that incubation of diesel exhaust particulate matter with both serum and lung cytosol extracted a substantial portion (79 - 85%) of the solvent-extractable mutagens. Although the serum-associated mutagens did not induce significant mutagenicity in Salmonella, incubation of the serum with protease increased the mutagenic activity of the serum, suggesting that the serum-extracted mutagens were bound to proteins and therefore unavailable to bind to Salmonella DNA under the assay conditions used by the authors. King *et al*. (1983) found that when lung macrophages engulfed whole diesel particles, the organic extract from the particles lost considerable mutagenic activity. The investigators hypothesized that alveolar macrophages are capable of removing mutagens from diesel particles and are capable of metabolizing the mutagenic compounds. Sun *et al*. (1988)

stated that the studies by Brooks *et al*. (1981) and King *et al*. (1981, 1983) "suggest that particle-associated organics become "bioavailable" to respiratory tract cells, allowing metabolic processes to occur".

In contrast to the earlier findings with simulated physiologic fluids, Wallace *et al*. (1987b) demonstrated that extraction with a phospholipid emulsion resembling a major component of lung surfactant showed substantial mutagenic activity, sometimes greater than that observed after extraction with organic solvents. The treatment of the fluid from the biological extraction process differed from the treatment of Siak *et al*. (1981), King *et al*. (1981) and Brooks *et al*. (1981). Wallace *et al*. kept the particles suspended in the extract whereas those particles were apparently removed in the earlier studies. Wallace *et al*. also showed that when the mixture was separated, the mutagenicity was in the sediment and not the filtered supernatant. Another complicating factor is that Wallace *et al*. scraped their soot from the exhaust pipe, thus allowing a different concentration of organic matter on the particles from that of the earlier samples obtained directly from the exhaust stream. However, a follow-up study by the same group (Keane *et al*., 1991) demonstrated similar results with either exhaust pipe soot or particles obtained directly from the exhaust stream. It should be noted that this method of diesel exhaust particulate matter presentation (e.g., keeping the particles suspended) is similar to that which would be expected to occur during an *in vivo* exposure, where diesel exhaust particulate matter would come into direct contact with alveolar epithelial cells or be phagocytized by alveolar macrophages.

Belisario *et al*. (1984) found that, with or without extraction, diesel exhaust particulate matter was directly mutagenic to four strains of S. typhimurium, but not to a fifth (TA-1535). Strains TA-98 and TA-1538 showed the highest activities. The mutagenicity occurred in the presence or absence of induced rat liver and whether or not the particles were extracted with dimethyl-sulfoxide. Those authors also showed clear dose-effect curves for TA-98 mutagenicity of the urine of Sprague-Dawley rats, collected within 24 hours of administration of diesel exhaust particulate matter in each of three ways: (1) injected intraperitoneally with corn oil or gelatin tablets, (2) injected subcutaneously in gelatin tablets or applied by gastric intubation, (3) suspended in a gum arabic solution with Tween. In contrast to the bacterial results, the activities of urine were greater (usually about two-fold) in the absence of metabolic activation than in its presence.

Whole diesel exhaust was also demonstrated by Courtois *et al*. (1993) to induce gene mutations in two strains of Salmonella (TA98 and TA100) directly exposed to a diesel exhaust stream in the absence of induced rat liver S9.

Schenker *et al*. (1992) determined the postshift mutagenicity in 87 railroad workers (306 samples) in various jobs. They used the sensitive microsuspension procedure with Salmonella strain TA-98, and they obtained the respirable-particle concentrations in work areas as a measure of diesel exhaust exposure. Smoking had a large effect on urinary mutagenicity, and after adjustment for smoking there was no independent association of respirable-particle concentration with the values of urinary mutagenicity that were measured.

Gu *et al*. (1992) examined the ability of diesel exhaust particles (DEP) to induce micronuclei *in vitro* in Chinese hamster V79 lung fibroblasts (V79) and Chinese hamster ovary (CHO) cells. DEP were suspended in either dimethyl sulfoxide (DMSO) or dipalmitoyl lecithin (DPL), a primary component of pulmonary surfactant. The suspensions were then separated into supernatant and sediment fractions, and the fractions were assayed for micronucleus induction (MI) in V79 and CHO cells. The DPL sediment fraction significantly increased MI at all concentrations tested in CHO cells, but only significantly increased MI in V79 cells at the middle concentration tested. The DMSO supernatant fraction significantly increased MI in both V79 and CHO cells at all concentrations tested. The finding that genotoxic activity was most strongly associated with the DPL sediment and DMSO supernatant fractions agrees with the findings of Wallace *et al*. (1987b) in Salmonella. This study is also discussed in section 5.2.3 (Micronucleus Assays) in greater detail.

5.1.3 MAMMALIAN CELL ASSAYS

Chesheir *et al*. (1981) demonstrated that diesel exhaust particles can cause mutations in a mammalian cell culture system as measured by forward mutation at the HGPRT locus in Chinese hamster ovary (CHO) cells. In these experiments, extraction was not necessary. The incubated cells readily phagocytized whole particles which subsequently became closely associated with the cell nucleus. With the same forward mutation assay, organic extracts from exhausts of five different diesel-powered vehicles demonstrated a weak mutagenic effect in CHO cells in the presence and absence of metabolic activation (Li and Royer, 1982). Casto *et al*. (1981) observed relatively weak to negative responses at the HGPRT gene locus measuring 6-thioguanine resistance in CHO cells treated with extract. Brooks *et al*. (1984) demonstrated mutations in CHO cells treated with extract in the presence but not in the absence of metabolic activation.

Studies of 8-azaguanine and ouabain resistance mutations in Chinese hamster V79 lung cells revealed that exhaust extracts from both light- and heavy-duty diesel engines exert a dose-dependent mutagenic effect. The light-duty samples were more mutagenic based on mutation frequency per µg diesel particulate extract. Mutagenic activity was lost after addition of the S15 metabolic activation system (Morimoto *et al*., 1986). The same investigators showed that both types of extracts induce 8-azaguanine resistant mutants in Syrian hamster embryo cells transplacentally exposed, thus correlating *in vivo* and *in vitro* findings.

Two separate studies found positive dose-related effects of extracts of diesel exhaust in the L5178Y mouse lymphoma assay (Mitchell *et al*., 1981; Rudd, 1979). The mutagenicity tended to be greater in the absence of metabolic activation. On the other hand, another team of investigators found that only in the presence of an exogenous activation system did dichloromethane extracts of diesel exhaust induce mutations in cultured human lymphoblasts (Liber *et al*., 1981). The activation system was a post mitochondrial supernatant derived from Aroclor-induced rat liver. Fractionation of the extract led to accounting for 44% of the mutagenicity by three PAHs: fluoranthene, 1-methyl phenanthrene and 9-methyl phenanthrene (Barfknecht *et al*. 1982).

In mouse BALB/c 3T3 cells, Curren *et al*. (1981) found that a dichloromethane extract of diesel exhaust particles from one light-duty diesel engine produced cell transformations and mutagenesis, as measured by ouabain resistance. Another light-duty engine and a heavy-duty engine did not produce the effect. Exogenous metabolic activation did not have a substantial effect on the results.

5.1.4 ONCOGENE AND TUMOR SUPPRESSOR GENE MUTATIONS

Pulmonary carcinomas from rats exposed by inhalation to diesel exhaust or carbon black were analyzed for mutations in the oncogene K-ras and the tumor suppressor gene p53 (Swafford *et al*., 1995; also in Belinsky *et al*., 1994). Increased levels of p53 protein were present in 1/2, 2/4 and 4/7 squamous cell or adenosquamous carcinomas from animals in the control, carbon black and diesel exhaust groups, respectively. However, no p53 mutations were noted in those neoplasms. Increased levels of p53 protein or p53 mutations were not noted in adenocarcinomas. Mutations in K-ras were only noted in 2 adenocarcinomas from the diesel exhaust-exposed group, and in 1 squamous cell carcinoma from the carbon black-exposed group. The authors noted that the K-ras gene in the rat is not always a target for genotoxic carcinogens.

5.1.5 GENE MUTATION IN YEAST

Lewtas and Williams (1986) reported that the most polar fraction of a diesel exhaust extract produces mutagenic and recombinogenic effects in *S. cerevisiae*, both with and without metabolic activation.

5.2 TESTS ASSESSING CLASTOGENICITY

Diesel exhaust particles or their extracts induce chromosome aberrations, aneuploidy, and sister chromatid exchange in rodent and human cells in culture. There is also evidence of micronuclei induction in mammalian cell systems.

5.2.1 CHROMOSOME ABERRATIONS

Hasegawa *et al*. (1988) investigated the ability of dichloromethane extracts of exhaust particles from light-duty and heavy-duty diesel engines to induce chromosome aberrations in cultured Chinese hamster V79 lung cells. The light-duty extracts induced a significant number of chromosome aberrations in the form of chromatid gaps and breaks whereas the heavy-duty extracts did not. Extracts from one light-duty engine induced structural abnormalities in Chinese hamster ovary (CHO) cells and human lymphocytes (Lewtas and Williams, 1986). In the presence of metabolic activation (rat liver S9), no abnormalities in the lymphocytes were observed. Organic extracts of diesel exhaust particulate with concentrations of extracted matter ranging from 30 to 250 µg/ml did not induce DNA strand breaks in Syrian hamster embryo cells in culture (Casto *et al*., 1981).

No chromosome damage detectable by metaphase analysis of femur marrow was observed in mice exposed by inhalation to diesel exhaust at 6 mg/m^3 for seven weeks (Pepelko and Peirano,

1983). No increase in the frequency of chromosome aberrations was noted in cultured lymphocytes from a group of 14 miners believed to have been exposed to diesel exhaust (Nordenson *et al*., 1981). After matching for age, smoking and length of time in their respective jobs, Fredga *et al*. (1982) found no significant difference in the incidence of chromosomal changes in workers exposed to diesel exhaust. There were no significant differences in frequency of aberrations or sister chromatid exchanges (SCEs) in the lymphocyte preparations. However, a statistically significant increase in the number of chromosome breaks was observed among the diesel exposed nonsmokers. The authors caution that this might be a random effect since 18 statistical tests were performed. They assessed significance with a sign test on each individual comparison. The IARC Working group noted that there were a small number of human subjects in each of these studies and, therefore, the negative results should be interpreted with caution (IARC, 1989).

5.2.2 SISTER CHROMATID EXCHANGE (SCE)

Investigators have demonstrated that extracts from diesel particles induce SCEs in Chinese hamster ovary (CHO) cells (Mitchell *et al*. 1981) and cultured human lymphocytes (Lockard *et al*., 1982). Further work with various extracts from light-duty and heavy-duty exhaust showed dose-dependent increases in the number of SCEs when applied to CHO cells and to cultured human lymphocytes (Hasegawa *et al*., 1988; Morimoto *et al*., 1986). In both of the cell systems tested, the light-duty samples were more potent.

Keane *et al*. (1991) studied the ability of diesel exhaust particles dispersed in aqueous dipalmitoyl phosphatidyl choline (DPL), a major pulmonary surfactant component, and of particle dichloromethane (DCM) extracts transferred to dimethyl sulfoxide (DMSO) to induce SCEs in Chinese hamster lung V79 cells without exogenous metabolic activation. The supernatant of the DCM/DMSO extract and the sediment of the DPL-dispersed particles both significantly increased SCE frequencies.

Cultured human lymphocytes obtained from healthy unrelated nonsmokers showed a 50% increase in SCE frequency (statistically significant, $p < 0.01$) in 2 of 4 donors after bubbling on-line diesel exhaust through the fluid above the lymphocytes (Tucker *et al*., 1986). The investigators commented that a lack of a mutagenic response in the cells obtained from the other 2 donors may be a result of differing individual sensitivities to diesel exhaust.

Several *in vivo* tests for SCE have been conducted. Following inhalation of 2 mg/m^3 of diesel exhaust for 35 hr/wk for 24 months, Lewis *et al*. (1986) observed no significant increases in SCE rates in lymphocytes from rats or monkeys. Bone marrow cells of rats inhaling diesel exhaust for 6 to 30 months at concentrations as high as 4 mg/m^3 showed no significant increase in SCE frequency (Morimoto *et al*., 1986).

Pepelko and Peirano (1983) compiled reports of studies sponsored by the U.S. EPA to investigate the effect of diesel exhaust particulate matter on the induction of SCE. Neither in Swiss Webster mice (Pereira *et al*. 1981b) nor in Chinese hamsters (Pereira, 1982) were SCEs in femur marrow elevated after breathing 6 mg/m^3 diesel exhaust for six months. Intraperitoneal

injection of diesel particulate (300 mg/kg body weight) or of the chloromethane extract from the particulate matter (500 mg/kg body weight) approximately doubled the observed number of SCEs in the femur marrow of $B6C3F_1$ mice relative to the DMSO controls (Pereira *et al*. 1981b), when observed two days after injection. The authors noted that the damage was not permanent since SCE frequencies in mice sacrificed 5 and 14 days post-treatment were not different from the DMSO controls. The same investigators showed that inhalation exposure of pregnant Syrian golden hamsters to whole exhaust from day 1 of gestation to day 12 (8 hr/day) did not induce an increase of SCE in fetal liver cells. Intraperitoneal injection of diesel exhaust particulate did not increase SCE frequency in fetal liver, but injection of the dichloromethane extracted matter resuspended in DMSO resulted in dose dependent increases.

In Syrian hamsters single intratracheal instillations of diesel particulate in the range of 0-20 mg produced a linear dose-response relationship for the number of SCEs observed from the finely minced cells of the lungs with trachea and bronchi removed. The instillations were in 0.25 ml of Hanks' balanced salt solution (Guerrero *et al*. 1981). Those investigators also found that inhalation of 6 mg/m^3 diesel exhaust for three months did not result in an elevation of lung SCEs in the Syrian hamsters, but exposure to 12 mg/m^3 for 3.5 months resulted in a doubling of lung SCEs.

5.2.3 MICRONUCLEI FORMATION

Gu *et al*. (1992) examined the ability of diesel exhaust particles (DEP) to induce micronuclei and alter phagocytosis *in vitro* in Chinese hamster V79 lung fibroblasts (V79) and Chinese hamster ovary (CHO) cells. DEP were suspended in either dimethyl sulfoxide (DMSO) or dipalmitoyl lecithin (DPL), a primary component of pulmonary surfactant. The suspensions were then separated into supernatant and sediment fractions, and the fractions were assayed for micronucleus induction (MI) and phagocytosis in V79 and CHO cells at concentrations of 34, 68 and 136 µg/ml. The DPL sediment fraction significantly increased MI at all concentrations tested in CHO cells, but only significantly increased MI in V79 cells at the middle concentration tested. The DPL supernatant fraction had no effect on MI in V79 cells, and only increased MI in CHO cells at the lowest concentration tested. The DMSO supernatant fraction significantly increased MI in both V79 and CHO cells at all concentrations tested. The DMSO sediment fraction had no effect on MI in CHO cells and only increased MI in V79 cells at the lowest concentration. The finding that genotoxic activity was most strongly associated with the DPL sediment and DMSO supernatant fractions agrees with the findings of Wallace *et al*. (1987b) in Salmonella. Increases in phagocytosis of DEP in the DMSO and DPL sediments occurred in both V79 and CHO cells in a dose-dependent manner.

No significant effects were obtained in the micronucleus assay performed on bone marrow cells from mice exposed for 6 months or rats exposed for 24 months to 2 mg/m^3 exhaust (Lewis *et al*., 1986). Micronuclei formation was not induced after inhalation exposure of mice to light-duty diesel engine exhausts at 0.4 mg/m^3 and 2.0 mg/m^3 for 4 to 18 months (Morimoto *et al*., 1986). A small but statistically significant ($p < 0.05$) increase in micronuclei formation was observed in erythrocytes of Chinese hamsters and mice exposed to 6 mg/m^3 exhaust for 6 months but not in an additional group of animals exposed to 12 mg/m^3 for one month (Pepelko and Peirano, 1983).

Intraperitoneal injection of either particulate or particulate extract did not result in increases in micronuclei as compared to the controls in hamsters observed from 30 hours to one week. In mice, micronuclei formation was doubled at the two highest dose levels after 30 hours, producing statistically significant increases compared to controls (Pereira *et al*., 1981a).

Odagiri *et al*. (1994) exposed cultured peripheral blood lymphocytes from 8 human donors to diesel engine exhaust particulate DCM/DMSO extracts from light duty (LD) and heavy duty (HD) engines. Antikinetochore antibody was used to distinguish between kinetochore-negative and -positive micronuclei, which indicates induction of clastogenicity and aneuploidy, respectively. A significant increase in the induction of kinetochore-positive micronuclei was noted in a majority of the cell samples treated with the highest dose tested (150 µg/ml) of LD extract. Some cell samples also demonstrated induction of kinetochore-negative micronuclei. Only one cell sample had significantly increased numbers of kinetochore-positive micronuclei after exposure to 400 µg/ml HD extract.

5.3 TESTS ASSESSING HERITABLE MUTATIONS

The possible induction of heritable mutations was measured in Drosophila using a sex-linked recessive lethal assay and in several multiple recessive strains of mice using heritable point mutations including induction of dominant lethals, induction of heritable translocations, oocyte killing and spermatogonial survival. In the battery of tests for heritable effects in mice, animals were exposed by inhalation to 6 mg/m^3 diesel exhaust particulate for up to 10 weeks. Findings in all of the aforementioned studies were negative (Pepelko and Peirano, 1983; Schuler and Niemeier, 1981). No significant effects were obtained in the dominant lethal assay performed in rats exposed by inhalation to 2 mg/m^3 of diesel exhaust for 6 months (Lewis *et al*., 1986).

5.4 TESTS ASSESSING PRIMARY DNA DAMAGE

Inhalation exposure to diesel exhaust results in unscheduled DNA synthesis *in vitro* in rodents and DNA adduct formation in rodents and monkeys. Increased levels of DNA adducts are associated with occupational exposure to diesel exhaust.

5.4.1 STUDIES IN MAMMALIAN CELLS AND ANIMALS

Suspensions of diesel exhaust particles added to cultured rat tracheal ring cells at particulate concentrations ranging from 0.125 to 2.0 mg/ml provoked unscheduled DNA synthesis (Kawabata *et al*., 1986). Repeated exposure of mouse embryo fibroblast C3H/10T1/2 cells with diesel particulate extracts in concentrations up to 50 µg/ml resulted in DNA adduct formation (Jeffrey *et al*., 1990). The statistical significance of these results was not stated.

The work of Wong *et al*. (1986) and Jeffrey *et al*. (1990) demonstrated a clear increase of DNA adduct formation in homogenate from lungs of rats exposed to diesel exhaust by inhalation for approximately 31 months to 7.1 mg/m^3 diesel exhaust particulate. Bond *et al*. (1989, 1990a, 1990b) studied the kinetics of formation and persistence of lung DNA adducts in rats and the effects of exposure concentration on DNA adduct formation. Rats exposed by inhalation to

diesel exhaust concentrations ranging from 0.35 to 10 mg/m^3 for up to 12 weeks and examined immediately thereafter had similar levels of DNA adducts in their lungs at the various doses. The number of DNA adducts seen in the exposed groups was about twice the number of adducts observed in the lungs of the control animals (statistically significant at $p < 0.05$). The investigators did not obtain a dose-response relationship. They suggested that at the concentration tested, the enzymes responsible for the formation of the metabolites that bind to DNA may have been saturated (Bond *et al*., 1990b). DNA adduct formation increased with increasing exposure duration and was highest at the end of the exposure period (12th week). The levels of adducts increased steadily after the fourth week of exposure, apparently nearing a steady state by the end of exposure. By the fourth week after the exposure ended the adduct levels decreased to control values.

Bond *et al*. (1988, 1990b) investigated location prevalence of the respiratory tract DNA adducts following exposure to diesel exhaust. The total level of DNA adducts was highest in the peripheral lung (excludes some bronchi) of rats that had been exposed to 10 mg/m^3 diesel soot for 12 weeks and examined immediately thereafter. Very few adducts were found in the major conducting airways. The frequency of occurrence of DNA adducts in nasal tissue was approximately 1/4 to 1/5 that seen in peripheral tissue. Further work demonstrated a 4-fold increase in the level of DNA adducts in alveolar type II cells of rats exposed for 12 weeks to 6.2 mg/m^3 diluted diesel exhaust as compared to sham-exposed controls (Bond *et al*., 1990c). The investigators noted that there are numerous other cell types in peripheral lung tissue, all of which may form DNA adducts after diesel exhaust exposure.

Because the site of tumors induced by diesel exhaust coincides with the region of the lung with the highest DNA adduct levels, Bond *et al*. (1990a,b) proposed the hypothesis that interaction of desorbed organic chemicals from diesel exhaust particles with lung DNA is a possible genetic mechanism for initiation of carcinogenesis. This hypothesis led the same investigators to study whether exposure to carbon particles not associated with mutagenic organic chemicals also elevates DNA adduct levels. Bond *et al*. (1990b) exposed rats by inhalation for 12 weeks to 0, 3.5 or 10 mg particles/m^3 of diesel exhaust or carbon black. The solvent extractable organic content of the diesel exhaust particles was about 30% as opposed to 0.04% for the carbon black particles. At the high dose level (10 mg/m^3) lung DNA adduct formation was found in both exposure groups and was 30% higher in diesel exhaust-exposed rats when compared to carbon black-exposed rats. At the lower dose level (3.5 mg/m^3), adducts were 50% above control values for diesel exhaust-exposed rats whereas no adduct formation was noted in the carbon black-exposed rats. Statistical significance was not reported by the investigators. The investigators suggested that the small amount of organic compounds associated with the carbon black particles at high doses may have been desorbed and metabolized to chemical forms that bind to DNA. In addition, the role of particles in diesel exhaust tumor induction may be related to their action as chronic inflammatory agents.

Bond *et al*. (1990b) also investigated species differences in peripheral lung DNA adduct formation. Mice, hamsters, rats and monkeys were exposed to diesel exhaust at 8.1 mg diesel exhaust particulate matter/m^3 for 12 weeks. Mice and hamsters had no increases of DNA adducts in their peripheral lung tissues. Rats and monkeys had 60-80% increase in peripheral lung DNA

adducts. These results reflect the carcinogenicity results to some degree. Hamsters have not exhibited positive responses for carcinogenicity and mice have been only marginally positive, with the filtered exhaust. Rats are strongly positive for carcinogenicity. The finding of a high level of adduct formation in monkeys suggests the plausibility of a high level of adduct formation in humans and the potential for carcinogenesis in humans.

Gallagher *et al*. (1990) treated mice topically with diesel-exhaust-particle extract and then determined DNA-adduct formation in skin, lung and liver using the ^{32}P-postlabeling assay in comparison to positive (BaP) and negative (acetone vehicle) controls. They found adduct formation for diesel exhaust, though not as much as for coke oven emissions and coal soot. Diesel exhaust produced a dose-dependent response in all three of the tissues examined.

Calf thymus DNA was exposed *in vitro* to diesel exhaust particulate matter extracts in the presence of either rat liver S9 (aerobic) or xanthine oxidase (XO)/hypoxanthine (anaerobic) (to detect reductive metabolites); one major adduct was noted with rat liver S9 using both nuclease P1 and butanol. This adduct also co-migrated with the major BaP-diol epoxide DNA adduct detected in skin DNA from mice and with the major adduct isolated from skin DNA from mice topically treated with DEP extract. Treating CT DNA with DEP extract and XO resulted in the formation of one major nuclease P1-sensitive adduct which was chromatographically different from the major DNA adduct observed in the rat liver S9-mediated incubations and from the major adduct detected in DNA from DEP extract-treated mouse skin. Based on its nuclease P1 sensitivity, and on chromatographic similarities to a major 1-nitropyrene-induced CT DNA adduct, the authors tentatively identified this adduct as an N-substituted arylamine adduct. Lung DNA from Cdl(WI)Br rats exposed to diesel exhaust containing 7.5 mg/m^3 DEP for 24 months demonstrated one major nuclease P1-sensitive adduct.

Gallagher *et al*. (1994) exposed Wistar rats to diesel exhaust (7.5 mg/m^3), carbon black (11.3 mg/m^3) or titanium dioxide (TiO_2) (10.4 mg/m^3) 18 hours/day, 5 days/week for 2 years; 2 and 6 month diesel exhaust exposure groups were also included. Lung DNA adduct levels were evaluated in control and treated animals using the ^{32}P-postlabeling assay. A nuclease P1-sensitive adduct, possibly resulting from nitro-PAH exposure, was elevated in diesel-exposed rats relative to the controls and not observed in rats exposed to carbon black or TiO_2. Additionally, the chromatographic properties of the adduct differed from dinitropyrene and 1-nitropyrene but were similar to nitrobenzo[a]pyrene and nitrochrysene.

The species differences in the incidence of adduct formation and the location of adducts within the respiratory tract are consistent with the pattern of tumor formation in exposed animals. However, rat DNA adduct levels were similar at all concentrations evaluated, and adducts were increased in rats at an exposure level (0.35 mg/m^3) that was not associated with increased tumor formation. The lack of a dose-response may be due to saturation of the metabolic pathway leading to adduct formation. The absence of tumors at the low exposure level could be due to an inadequate number of animals. As Bond *et al*. (1990a) pointed out, it is likely that factors in addition to lung DNA-adduct formation are involved in carcinogenicity induced by diesel exhaust. The formation of lung DNA adducts by metabolites of particle-associated organic

compounds may only be one step in the initiation of diesel exhaust-induced pulmonary carcinogenesis.

Lung DNA samples from male and female F344 rats exposed to diesel exhaust or carbon black (16 hours/day, 5 days/week) at concentrations of 2.5 or 6.5 mg/m^3 for 3, 6, 12, 18 or 23 months (Mauderly *et al*., 1994; also in Nikula *et al*., 1995) were analyzed for DNA adducts using the ^{32}P-postlabeling assay (Randerath *et al*., 1995). Exposure-specific DNA adducts were not observed in either the diesel exhaust or carbon black exposure groups. Some quantitative alterations of I-compounds, which have been hypothesized to be DNA modifications of endogenous origin were observed; total DNA adducts in the 6.5 mg/m^3 diesel exhaust group were significantly elevated after 3 months of exposure. However, this increase was not noted for other time points.

Savela *et al*. (1995) used an HPLC separation/^{32}P-postlabeling assay detection method to analyze DNA adducts formed by diesel exhaust extracts incubated with calf thymus DNA in the presence of rat liver S9 *in vitro* or in mouse skin and lung after *in vivo* topical treatment. Diesel exhaust extract-induced adducts were found in all three DNA sources. This study suggests that dermal exposure to diesel exhaust may result in the occurrence of DNA adducts in lung and other organs distant from the site of exposure.

5.4.2 STUDIES IN HUMANS

In vitro treatment of human lymphocytes with diesel exhaust particle extract resulted in the detection of five DNA adducts using the ^{32}P-postlabeling assay (Gallagher *et al*., 1993). One adduct comigrated with the major adduct detected in human lymphocytes treated with benzo[a]pyrene (BaP).

Hemminki *et al*. (1994) compared levels of aromatic DNA adducts by the ^{32}P-postlabeling assay in peripheral blood lymphocytes isolated from nonsmoking bus maintenance and truck terminal workers; hospital mechanics were used as a control group. Diesel mechanics and garage workers in the bus maintenance worker group could have been exposed to PAHs through dermal exposure to used lubricating oil and diesel fuel, respectively. Adduct levels in all bus and truck terminal workers were elevated when compared to controls. The highest adduct levels in the bus maintenance workers were noted in the garage workers (3.63 adducts/10^8 nucleotides, compared to 2.08 adducts/10^8 nucleotides in controls); the highest adduct levels in the truck terminal workers were noted in the diesel forklift workers (3.73 adducts/10^8 nucleotides). Diesel exhaust exposure levels were not available for the workplaces at the time of the study.

The relationships between DNA adduct levels and hypoxanthine-guanine phosphoribosyl transferase (hprt) mutant frequencies in T-lymphocytes and the genotypes for glutathione transferase (GSTμ) and N-acetyltransferase (NAT2) in non-smoking bus maintenance workers exposed to diesel exhaust were examined by Hou *et al*. (1995). The garages used as the source of the bus maintenance workers were apparently the same as those used in the study by Hemminki *et al*. (1994); as noted previously, diesel exhaust exposure levels were not available for the workplaces at the time of the study, and diesel mechanics and garage workers in the bus maintenance worker group could have potentially been exposed to PAHs through dermal

exposure to used lubricating oil and diesel fuel, respectively. DNA adducts were quantified using the ^{32}P-postlabeling assay. Highly significant differences ($p = 0.0009$) were observed between the diesel exhaust-exposed workers and the controls (3.2 and 2.3 adducts/10^8 nucleotides, respectively). No difference in hprt mutant frequency was observed between the 47 exposed and 22 control individuals; however, both mutant frequency and adduct level were highest in the 16 most heavily exposed workers. Increased mutant frequency correlated significantly with increased adduct levels. No significant difference was observed in either mutant frequencies or DNA adduct levels between the GSTM1-negative and positive individuals, or between the slow and rapid acetylators. However, among the slow acetylators, GSTM1-negative individuals demonstrated significantly higher adduct levels when compared to GSTM1-positive individuals.

Lymphocyte DNA adducts, hemoglobin adducts, and urinary 1-hydroxypyrene (1-HP) levels were studied in bus garage workers and mechanics exposed to diesel exhaust and in appropriate controls by Nielsen *et al*. (1996). DNA adducts were assayed using the ^{32}P-postlabeling assay. Total DNA adducts were significantly increased in diesel-exposed workers compared to controls [0.84 fmol/µg DNA vs. 0.26 in controls (butanol extraction); 0.65 fmol/µg DNA vs. 0.08 in controls (P1 nuclease isolation)]. Median hemoglobin (hydroxyethylvaline) adducts in diesel-exposed workers were 33.3 pmol/g hemoglobin compared to 22.1 pmol/g in controls. Urinary 1-HP levels in exposed workers and controls were 0.11 and 0.05 µmol/mol creatinine, respectively. The authors stated that the study data indicate that skin absorption of PAH might be an important factor to consider when studying PAH exposure from air pollution sources. Additionally, as noted for the studies by Hemminki *et al*. (1994) and Hou *et al*. (1995), diesel mechanics and garage workers in the bus maintenance worker group could have been exposed to PAHs through dermal exposure to used lubricating oil and diesel fuel, respectively. The authors stated that in contrast to the findings by Hemminki *et al*. (1994), DNA adduct levels were higher in bus mechanics than in the garage workers; however, the authors did not provide information on whether that difference is statistically significant, nor did they give quantitative levels for those two groups as opposed to the combined levels provided in the article. Additionally, Nielsen *et al*. (1996) state that "it appears from the description of working conditions that the garage workers in the Swedish study were more exposed than the individuals in our study", which could account for mechanics displaying higher levels of DNA adducts than garage workers in their study.

DNA adducts in peripheral blood mononuclear cells obtained from coal mine workers exposed to diesel exhaust were evaluated using the ^{32}P-postlabeling assay by Qu *et al*. (1997). Blood samples were taken at two different mines (Mines A and B), before and after a period of intense diesel exhaust exposure (long wall change out, LWCO). Participants were classified into 5 specific job categories: Job 1 (fitters), Job 2 (loadmen and miners), Job 3 (deputy, underground managers, shift managers, engineers, electricians and surveyors), Job 4 (machinemen, drivers, shiftmen and mechanical unit) and Job 5 (clerks, surfacemen, lamp cabin attendants, planning coordinators, safety training coordinators and boiler makers). Workers in job category 5 were considered to not be exposed to diesel exhaust. A total of 89 and 75 workers were studied at Mines A and B, respectively. Follow-up data after LWCO were available for 7 and 61 workers at Mines A and B, respectively. DNA samples taken from the same individual before and after

LWCO were analyzed on the same chromatography plate in order to reduce the effect of plate to plate variability. Data from the two mines were analyzed separately.

Applying univariate linear regression models to Mine A DNA adduct data demonstrated a negative association between total adducts and length of time on the job, and a positive association with smoking status and job category. There was no significant association between total DNA adducts and reported exposure level. However, workers in the more highly exposed job categories 2 and 4 had total adduct levels approximately 60% greater than the non-exposed surfacemen (Job 5). Application of multivariate linear regression models provided similar results. Six of seven workers evaluated for adducts after LWCO demonstrated increases in total DNA adducts; the geometric mean of those adduct levels increased from 330 to 456 attamoles/µg DNA (38% increase).

Analysis of Mine B total DNA adduct data did not reveal any significant association between adducts and smoking status, length of time on the job, exposure category or job classification. Total adduct levels were increased in the exposed job classifications compared to the nonexposed classifications (approximately 2-fold), but were not statistically significant. Workers evaluated for adducts after LWCO demonstrated a statistically significant increase (32%) in total DNA adducts as a group. Adjustment for reported exposure level, smoking status, job category and time on job had little effect on the estimated effect.

5.5 TESTS ASSESSING OXIDATIVE DNA DAMAGE

5.5.1 ACTIVE OXYGEN GENERATION

Generation of hydrogen peroxide or active oxygen species (superoxide anion, hydroxyl radicals) by environmental chemicals or endogenous oxidation processes may result in damage to cellular DNA. Reaction of DNA with hydroxyl radicals has been demonstrated to cause the formation of the modified bases thymine glycol and 8-hydroxydeoxyguanosine (8-OHdG). Formation of 8-OHdG adducts leads to G:C to T:A transversions unless repaired prior to replication, and therefore may be promutagenic (Nagashima *et al*., 1995). Inhibition of cellular hydrogen peroxide and active oxygen detoxifying enzymes (catalase, glutathione peroxidase, superoxide dismutase) may also result in oxidative DNA damage (Budroe and Williams, 1994).

Diesel exhaust particles were shown by Sagai *et al*. (1993) to produce superoxide and hydroxyl radicals *in vitro* in the absence of a biological activation system. Superoxide and hydroxyl radical production were inhibited by superoxide dismutase (SOD) and dimethyl sulfoxide (DMSO), respectively. Methanol-washed diesel exhaust particles did not produce superoxide and hydroxyl radicals, indicating that the active components were extractable with organic solvents. Lung antioxidant enzyme (SOD, glutathione-S-transferase, glutathione peroxidase) activities were significantly reduced in mice exposed to diesel exhaust particles by intratracheal instillation. Additionally, instillation of 1 mg of diesel exhaust particles caused 100% mortality within 24 hours; in contrast, the mortality in diesel exhaust particle-exposed mice pretreated either with polyethylene glycol-conjugated SOD or a radical scavenger, butylated hydroxytoluene (BHT) was significantly reduced, and treatment of mice with 1 mg of methanol-washed diesel exhaust

particles was not lethal at 24 hours. These data suggest that diesel exhaust particles can induce the production of active oxygen species and decrease antioxidant enzyme activities *in vivo* in mice.

Exposure of microsomal and cytosolic fractions of mouse lung to a methanol extract of diesel exhaust particles (DEP) resulted in increased oxidation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) in the microsomal fraction; less NADPH oxidation was seen in the cytosolic fraction (Kumagai *et al*., 1997). This indicated that DEP contain substrates for NADPH-cytochrome P450 reductase rather than DT-diaphorase. Use of purified P450 reductase as the enzyme source resulted in a 260-fold increase in turnover. A DEP methanol extract caused a significant production of superoxide in the presence of P450 reductase; electron spin resonance (ESR) determinations indicated that hydroxyl radical was also formed. The active oxygen species generated by DEP/P450 reductase were found to cause DNA strand breaks *in vitro*. Addition of DMSO or desferal (which inhibit hydroxyl radical production), SOD or catalase to the reaction mixture resulted in reduced DNA damage. The authors concluded that DEP components, probably quinoids or nitroaromatics, appear to promote DNA damage through redox cycling-based superoxide generation.

Catalase is released by alveolar cells into lung surfactant fluid. Inhibition of catalase activity would result in reduced detoxification of hydrogen peroxide, and could result in increased oxidative DNA damage. Mori *et al*. (1996) incubated saline extracts of diesel exhaust particles with catalase from bovine liver or guinea pig red blood cells or alveolar cells in an *in vitro* reaction system. The extracts caused a significant dose-dependent decrease in catalase activity from all sources.

5.5.2 OXIDATIVE DNA ADDUCT FORMATION

Seto *et al*. (1994) demonstrated that diesel exhaust particle extracts were capable of oxidizing 2′-deoxyguanosine to 8-OHdG in an *in vitro* reaction system.

The incubation of diesel exhaust particles with calf thymus DNA *in vitro* resulted in the formation of 8-OHdG adducts (Nagashima *et al*., 1995). Exposure of ICR male mice to diesel exhaust particles by intratracheal instillation resulted in a significant increase (approximately 3-fold) in mouse lung DNA 8-OHdG adducts.

Male ICR mice were exposed by intratracheal instillation (one treatment/week for 10 weeks) to 0.1 mg of either titanium dioxide (TiO_2), DEP, or hexane/benzene/methanol-washed DEP (WDEP) (Ichinose *et al*., 1997b). Statistically significant increases in mouse lung 8-OH-dG DNA adducts compared to controls were observed in the WDEP and DEP treatment groups (142 and 179% of control, respectively), but not in the TiO_2 group (109% of control).

5.6 TEST ASSESSING CELL TRANSFORMATION ABILITY

Particulate extracts from a light-duty diesel engine showed cell transforming ability in mouse BALB/c 3T3 cells in the absence but not in the presence of metabolic activation (Curren *et al*., 1981). In the same study, particulate extracts from an Oldsmobile diesel engine and a Caterpillar diesel engine showed essentially no activity. Extract samples from three of four diesel engines enhanced transformation of Syrian hamster embryo cells in the presence of the SA7 virus (Casto *et al*., 1981). The viral enhancement assay measures the increased sensitivity of cells to virus-induced transformation. Although this assay is listed as a transformation assay, it may be a measure of DNA damage (Lewtas and Williams, 1986).

5.7 TESTS ASSESSING *Ah* RECEPTOR BINDING

Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been demonstrated to result in a number of toxic effects, including induction of carcinogenicity in animals. Many of the toxic effects of TCDD and other chemicals possessing TCDD-like activity are believed to be mediated via an intracellular protein (the *Ah* receptor). The *Ah* receptor is a ligand-dependent transcription factor; this indicates that the mechanism for at least some of the toxic effects elicited by chemicals possessing TCDD-like activity reflect sustained alterations in gene expression. Several studies have found that diesel exhaust particulate extracts or the semivolatile phase of diesel exhaust can bind to the *Ah* receptor, suggesting that some of the toxic effects of diesel exhaust may be mediated by the *Ah* receptor.

Mason (1996) examined the dioxin receptor (*Ah* receptor) binding activity of diesel exhaust particle extracts using an *in vitro* hydroxylapatite ligand binding competition assay. Relative binding affinities were expressed as IC_{50}, which is the amount of sample required to reduce the specific binding of 1 pmole/ml [^{3}H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the *Ah* receptor by 50%. The diesel exhaust particle extracts tested were found to have an IC_{50} (expressed as activity per driving distance) of 0.002 miles/ml. The diesel exhaust particle extracts were also assayed for AHH induction as a marker of *Ah* receptor binding activity in the rat hepatoma cell line H4IIE. Extracts were fractionated on the basis of increasing polarity into five fractions (I to V). More than 85% of the *Ah* receptor binding activity was found in fraction III (nitro-PAHs), and about 10% was found in fraction II (heavy aliphatic hydrocarbons and PAHs); the remaining activity was primarily found in fraction IV (dinitro-PAHs and quinones). The *Ah* receptor binding activity of the semivolatile phase of bus diesel exhaust was also described. A significant amount of *Ah* receptor binding was observed; binding affinities differed depending on the particular diesel engine fuel used to generate the emissions.

A partial least squares regression analysis technique was used by Sjögren *et al*. (1996) to examine the relationship between diesel fuel physical and chemical characteristics and diesel exhaust particle extract mutagenicity and *Ah* receptor binding. The ligand binding competition assay described above was used to determine *Ah* receptor binding affinities. Cetane number and upper distillation curve points were negatively correlated with *Ah* receptor binding, and 1-nitropyrene and indeno[1,2,3-cd]pyrene content, particle-bound nitrate and emitted mass of particles were positively correlated with *Ah* receptor affinity.

5.8 SUMMARY OF GENOTOXIC EFFECTS

Diesel exhaust particles or their extracts are mutagenic in bacteria (*S. typhimurium* and *E. coli*). Results of the Salmonella reverse mutation assay showed that nitro-PAHs are the principal mutagenic species and that fuel, engine type, operational characteristics of the vehicles and ambient conditions influence the mutagenicity of diesel engine exhaust. Early investigations of the ability of physiologic fluids to extract mutagens from diesel exhaust particles gave weakly positive or negative results. More recent laboratory techniques which more closely model *in vivo* exposure conditions have often been able to make available greater mutagenic activity than that observed with organic solvent extraction.

Diesel exhaust particles or their extracts are mutagenic in several mammalian cell systems (CHO, V79, BALB/3T3, human lymphoblasts). Unlike the mixed findings obtained with nonhuman mammalian cells, mutagenic effects in human lymphoblasts resulted only with addition of a metabolic activation system.

Exhaust particles or their extracts induce chromosome aberrations, aneuploidy and SCEs in rodent and human cells in culture. Most *in vivo* tests for SCEs, germ cell mutations, micronuclei formation and dominant lethals in rodents exposed by inhalation to diesel exhaust gave weakly positive or negative results. As pointed out by IARC (1989) no study adequately evaluates whether exposure to diesel exhaust induces chromosomal effects in humans, because of the limited sample sizes of those studies.

In vitro exposure to diesel exhaust particulate matter can cause unscheduled DNA synthesis in mammalian cells. Exposure by inhalation to diesel exhaust can result in DNA adduct formation in rodents and monkeys, particularly in peripheral lung cells. Human studies also indicate that increased levels of DNA adducts are associated with occupational exposure to diesel exhaust, and that increased levels of T lymphocyte mutations (hprt locus) are correlated with increased levels of diesel exhaust-induced T lymphocyte DNA adducts. Formation of lung DNA adducts by organic chemicals or their metabolites desorbed from diesel exhaust particles or contained in the semivolatile exhaust phase may provide a step in the initiation of pulmonary carcinogenesis induced by diesel exhaust.

The studies described above which indicate that human exposure to diesel exhaust results in the formation of DNA adducts support the results of epidemiological studies which describe a positive correlation between human diesel exhaust exposure and the induction of lung cancer. Diesel exhaust particles or their extracts are also capable of inducing cell transformation.

Diesel exhaust particles or their extracts have been demonstrated to produce superoxide and hydroxyl radicals and inhibit the hydrogen peroxide detoxifying enzyme catalase *in vitro,* and inhibit the antioxidant enzymes superoxide dismutase, glutathione-S-transferase and glutathione peroxidase in mouse lung *in vivo* after intratracheal instillation. Diesel exhaust particles have also been shown to cause an increase in 8-hydroxydeoxyguanosine (8-OHdG) adducts in calf thymus DNA *in vitro* and in lung DNA from mice exposed *in vivo* by intratracheal instillation.

Both diesel exhaust particle extracts and the semivolatile phase of diesel exhaust have been shown to have dioxin receptor (A*h* receptor) binding affinity. The physical and chemical characteristics of diesel fuel have been demonstrated to affect the A*h* receptor binding affinity of the resulting diesel engine emissions.

6.0 CARCINOGENIC EFFECTS

6.1 ANIMAL STUDIES

This section discusses the many diesel exhaust animal cancer bioassays. It is organized by route of exposure, with the primary emphasis on inhalation studies. Inhalation is the most common pathway for diesel exhaust exposure in the experimental studies. Studies of intratracheal administration of diesel exhaust components are closely related, so they are also reviewed. Although mucociliary clearance routinely transports deposited materials from the airways to the gastrointestinal tract, there are no available studies by oral administration of whole or fractionated diesel exhaust. Studies of skin painting are reviewed since skin painting of diesel exhaust extracts allows a relatively simple screening of components and could suggest the carcinogenic mechanism; additionally, studies suggest that dermal exposure to diesel exhaust may result in an increased risk of genotoxicity (Savela *et al*., 1995; Nielsen *et al*., 1996) and therefore possibly carcinogenicity. Further comprehensive reviews may be found in IARC (1989), HEI (1995) and WHO (1996).

Until the early-1980's, inhalation studies in rodents examining the potential carcinogenic effect of diesel exhaust emissions failed to demonstrate any statistically significant increase in the incidence of pulmonary tumors in exposed animals. More recent studies utilizing higher exposure levels and/or longer observation periods (>24 months) have consistently demonstrated significant increases in pulmonary tumors in rats. Unfiltered diesel exhaust is tumorigenic in the rat lung in a number of studies at exposures of 2.2 mg/m^3 or greater (time-weighted average equivalent to 1.05 mg/m^3) for varied daily durations over 2 years. Nonsignificant increases in lung tumor incidence in rats have been observed at diesel exhaust particulate concentrations between 0.35 and 2.2 mg/m^3. Studies in mice have mixed results. Unfiltered diesel exhaust significantly increased lung tumor incidence in female Strong A mice, female Sencar mice, and female NMRI mice. In female Strong A mice, however, the highest exposure resulted in a decrease in lung tumor incidence relative to controls. Exposure of female NMRI mice to filtered diesel exhaust has produced both positive and negative results. Other studies in mice are negative. Negative results in hamsters are consistent with the finding that, unlike rats, hamsters do not demonstrate increases in DNA adduct formation following a 12-week exposure to diesel exhaust particulate matter (see Section 5.4).

6.1.1 INHALATION OF DIESEL EXHAUST OR DIESEL EXHAUST COMPONENTS ALONE

Tables 6.1 a-c summarize the experimental inhalation studies of diesel exhaust (or diesel exhaust components) carcinogenicity. Three animal species -- rats, mice and Syrian hamsters -- have been adequately studied for the carcinogenic effects of inhalation of diesel exhaust. Studies in rats and mice have been positive and are therefore discussed below in detail. As studies in hamsters yielded only negative results, they are discussed briefly and are described in Table 6.1a. A study by Lewis *et al*. (1986; 1989) in cynomolgus monkeys was negative. The duration of exposure in Lewis *et al*. (1986) was considerably less than lifetime, rendering it unsuitable for

a determination of carcinogenicity. However, since it is the only non-human primate chronic diesel exhaust exposure study, it is also discussed in detail.

Studies examining the effect of diesel exhaust inhalation on the potency of known carcinogens administered by other routes, either injection or intratracheal instillation are also described in this chapter. In the inhalation studies described, animals were exposed to whole diesel exhaust unless otherwise noted. Concentrations of diesel exhaust are expressed as the particulate matter level in the exhaust. For example, an exposure to 6 mg/m^3 diesel exhaust means that the animals were exposed to whole diesel exhaust containing 6 mg/m^3 particulate matter.

6.1.1.1 STUDIES IN MICE:

The carcinogenicity of diesel exhaust in mice has been evaluated by Pepelko and Peirano (1983), Heinrich *et al*. (1986a), Takemoto *et al*. (1986), Heinrich *et al*. (1995), and Mauderly *et al*. (1996). Each study is summarized below. (See also Table 6.1.b)

Pepelko and Peirano, 1983:

The effects of diesel engine emissions were evaluated in three different groups of mice: Strong A, Jackson A, and Sencar. Strong A and Jackson A were evaluated using the Strain A mouse pulmonary adenoma assay. Requiring only seven to eight months of exposure, this is basically a screening test for carcinogens. A negative result does not necessarily rule out the possibility that the test substance may be carcinogenic. The protocol involved examining the surface of excised lung lobes for white nodules. These adenomas are benign tumors. There is probably insufficient time for them to progress to the stage of malignancy in such a short duration of exposure. Strong A mice were exposed for 8 or 20 h/d, 7 d/w, to clean air, diesel exhaust containing particulate at levels of 6 mg/m^3, or diesel exhaust container 12 mg particulate/m^3 starting at 6 weeks of age. Sample size ranged from 25 to 430 (see Table 6.1.b). Jackson A mice (20 males, 20 females) were exposed to clean air or 6 mg/m^3 diesel exhaust. The exposure periods varied from 8 weeks to 46 weeks in length and animals were kept for varying lengths of time after exposure ceased. Results for a matched group of animals also injected with a single dose of 1 or 5 mg urethane at the start of exposure will be discussed in Section 6.1.1.3. Diesel exhaust was generated using a 6 cylinder Nissan engine run on the Federal Short Cycle. Particle diameters were generally < 0.1 µm. The analytical methods used to characterize the particles were not reported.

In the Jackson A mice, the clean air and diesel exposed (6 mg/m^3) animals exhibited similar tumor incidence. In male Strong A mice there were no significant differences between clean air and 6 mg/m^3 diesel exposed animals. In female Strong A mice there was a small but statistically significant increase in pulmonary tumor incidence in the group exposed to 6 mg/m^3 diesel exhaust compared to those breathing clean air. (See Table 6.2.b. and Section 6.1.1.3 for description of this positive study.)

In contrast, the exposure of Strong A mice to 12 mg/m^3 diesel exhaust resulted in a significant decrease in tumors in both females and males compared to clean air control animals (22 with tumors/258 mice exposed versus 59 with tumors/250 control mice). The authors could not

explain the inconsistent results produced at the different exposure levels, but noted that the results could not be explained by increased mortality of the mice susceptible to tumor induction because survival rates were not significantly altered by exposure conditions.

The carcinogenic effects in Sencar mice (130 males, 130 females) were examined in a two-generation study. The parent generation was exposed continuously to clean air or 6 mg/m^3 diesel exhaust from weaning age to sexual maturity and then mated. Exposure of the dams was continued through pregnancy, parturition, and weaning of offspring. The concentration was increased to 12 mg/m^3 when the mean age of the offspring was 12 weeks and continued until 15 months of age, when the study was terminated.

The body weight of diesel exposed animals was depressed after 40 weeks of exposure. The percent of pulmonary tumors ($p<0.05$), particularly adenomas ($p<0.02$), was significantly increased in the female offspring exposed to diesel exhaust. No significant differences were detected in the male offspring exposed to diesel exhaust.

Heinrich *et al.*, 1986a:

Female NMRI mice were exposed for 19 h/d, 5 d/wk for up to 120 weeks. All animals were 8 - 10 weeks old at the start of exposure. The animals were placed in one of three exposure groups of 96-animals each: 1) clean air; 2) filtered diesel exhaust (i.e. without the particles); and 3) unfiltered diesel exhaust. Filtered and unfiltered exhaust were diluted 17-to-1. Particulate concentration was approximately 4 mg/m^3 in the unfiltered diesel exhaust exposure. A 40 kilowatt 1.6 liter diesel engine served as the source for the exhaust emissions. The engine was operated continuously according to the US 72 test driving cycle. The diesel fuel used was a European Reference Fuel with a sulfur content of 0.36%. The mass median aerodynamic diameter (MMAD) of the diesel particles was approximately 0.35 µm. Histological evaluations of 12 different types of tissues, or in some cases an additional eight different types, were conducted at scheduled or moribund sacrifices.

The body weights of the mice exposed to the filtered exhaust did not significantly differ from the controls. The body weights of mice exposed to unfiltered diesel exhaust fell significantly below control values after approximately 480 days. After this time the exposed mice suffered from a significantly higher weight loss than the controls. An exposure-related elevation of mortality rate occurred with mice after two-years exposure to unfiltered diesel exhaust. After the two years of exposure to unfiltered diesel exhaust the wet weight and dry weight of the lungs was increased to four and seven times higher than the controls.

The control group of mice showed a spontaneous lung tumor incidence of 13% at the end of the study. Exposure to unfiltered and filtered diesel exhaust for 120 weeks increased the tumor incidence at the end of the study to 32 and 31%, respectively. The numbers surviving in the three groups were 84, 93 and 76 out of 96 entering the study. Exposure to both filtered and unfiltered diesel exhaust caused a significant increase in adenocarcinomas, while the adenoma incidence was practically the same as in the controls. Spontaneous adenocarcinomas of the lung were found in 2.4% of the control mice. No effects could be observed in the upper respiratory

tract (i.e., nasal cavities, larynx or trachea). Both filtered and unfiltered diesel exhaust increased the incidence of malignant lung tumors.

Takemoto *et al*., 1986:

Newborn male and female C57BL/6N and ICR mice were exposed to diesel exhaust (2 - 4 mg/m^3 particulate concentration) or clean air for 4 h/d, 4 d/wk starting within 24 hours of birth and continuing for up to 28 months. Necropsies were carried out at 3, 6, 12, 18 and 28 months on 114, 85, 72 and 44 ICR mice and 50, 46, 98 and 103 C57BL mice, respectively. Exhaust was produced by a small diesel engine (YANMAR NSA-40CE) normally used for an electric generator. The authors stated that the engine had some different characteristics from those of a diesel-powered car; however, these characteristics were not reported. The MMAD of the particles was 0.32 µm.

No lung tumors were detected in control or diesel exposed C57BL/6N mice necropsied up to 12 months. In the 13-to-18 month and 19-to-28 month periods combined (male and female mice), adenomas or adenocarcinomas were found in 17/150 (11%) of exposed mice, compared to 1/51 (0.02%) in controls.

In ICR mice no lung tumors were detected until after 12 months. In the 13-to-18 month and 19-to-28 month periods combined (male and female mice), adenomas or adenocarcinomas were found in 14/56 (25%) of exposed mice, compared to 7/60 (12%) in controls.

In this study the authors stated that none of the individual increases in the lung tumor incidence of diesel exhaust exposed mice was statistically significant compared with control animals breathing clean air. However, a statistical analysis by Pott and Heinrich (1990) indicated that the difference in benign and malignant tumors between diesel exhaust-exposed C57BL/6N mice and the corresponding controls was significant at $p < 0.05$. Diesel exhaust exposure did not result in any statistically significant increase in tumors in seven other organs examined.

Heinrich *et al*., 1995:

Female NMRI mice were exposed to diesel exhaust containing 7 mg/m^3 particulate matter, carbon black (7.4 mg/m^3 for 4 months, followed by 12.2 mg/m^3 for 9.5 months), or titanium dioxide (TiO_2) (7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 5.5 months) for 13.5 months, followed by clean air for 9.5 months. A second set of female NMRI mice were exposed to diesel exhaust containing 4.5 mg/m^3 particulate matter or the equivalent concentration of diesel exhaust with the particulate matter removed by filtration (particle-free diesel exhaust) for 23 months. Appropriate clean air controls were included. 80 and 120 animals/group were examined for tumors in the 13.5 and 23 month exposure durations, respectively. Female C57BL/6N mice were exposed to diesel exhaust containing 4.5 mg/m^3 particulate matter or the equivalent concentration of particle-free diesel exhaust for 24 months, followed by clean air for 6 months. 120 animals/group were examined for tumors. Diesel exhaust was generated by two 1.6 L Volkswagen diesel engines. One engine (the primary exhaust source) was operated on the U.S. 72 cycle; when necessary, exhaust gas was

supplemented by the second engine, which was operated under constant load conditions (2500 U/minute, 40 N). The mass median aerodynamic diameter (MMAD) of the exposure chamber particles were 0.25, 0.64 and 0.80 µm for diesel exhaust, carbon black and TiO_2, respectively. All animals were 7 weeks of age at the start of exposure, and were exposed for 18 hours/day, 5 days/week. The carbon black and TiO_2 exposure concentrations were varied to maintain a lung particle load comparable to the diesel exhaust exposure group as determined from particle lung burden data (obtained at 3, 6, and 12 months of exposure).

The mortality rates for the NMRI mice exposed to treatments for 13.5 months were 10% (controls), 16% (diesel exhaust), 20% (carbon black) and 30% (TiO_2) at the end of treatment. A 50% mortality rate was reached at 17, 19, 19 and 20 months of age for the TiO_2, diesel exhaust, carbon black, and control groups, respectively. Mean body weights of the diesel exhaust, carbon black and TiO_2 exposure groups were significantly less (5-7%) than those of controls after 6 months, 11 months and 8 months, respectively. Mean lung wet weights at 3 and 12 months of exposure were increased in the diesel exhaust (0.3 g, 0.6 g), carbon black (0.3 g, 1.0 g) and TiO_2 (0.3 g, 0.9 g) treatment groups compared to controls (0.2 g, 0.2g). Mean lung particle burdens (mg/lung) after 3, 6 and 12 months of exposure were: 1.7, 4.1 and 7.0 for the diesel exhaust exposure group; 0.8, 2.3 and 7.4 for the carbon black exposure group; and 0.8, 2.5 and 5.2 for the TiO_2 exposure group, respectively. The mean lung particle loads after 12 months of exposure (expressed as mg particles/g clean air control lung) in the diesel exhaust, carbon black and TiO_2 exposure groups were 35, 37 and 26 mg, respectively. The adenoma/adenocarcinoma percent incidence in those exposure groups was 21.8%/15.4% (diesel exhaust), 11.3%/10% (carbon black), 11.3%/2.5% (TiO_2) and 25%/15.4% (clean air controls). The lung tumor rates (adenomas and adenocarcinomas combined) for the diesel exhaust (32.1%), carbon black (20%) and TiO_2 (13.8%) exposure groups were not significantly different from that of the control group (30%). A tumor rate analysis which compensated for the significantly increased mortality of the treatment groups compared to controls at 13.5 months also found that lung tumor rates in the treatment groups were not significantly increased when compared to controls.

The authors stated that mortality rates in NMRI mice exposed for 23 months were only slightly higher for 4.5 mg/m^3 diesel exhaust-exposed mice starting at 350 days (from birth) compared to the control and particle-free exhaust groups. Diesel exhaust and particle-free exhaust treatment group mean body weights were significantly decreased starting at 1 year and continuing to the end of the exposure period. Mean lung wet weights of the diesel exhaust-exposed group were 2-fold and 3-fold greater than controls after 6 and 18 months, respectively. Mean lung wet weight data was not provided for the particle-free exhaust treatment group. Mean lung particle burdens after 3, 6, 12 and 18 months of exposure were 0.9, 2.4, 4.0 and 5.9 mg/lung for the diesel exhaust exposure group. The mean lung particle load after 18 months of exposure (expressed as mg particles/g clean air control lung) was 29.5 mg. The control, diesel exhaust, and particle-free exhaust groups demonstrated percent incidences of adenomas/adenocarcinomas of 25%/8.8%, 18.3%/5% and 31.7%/15%, respectively. The total tumor percent incidence (benign and malignant combined) was 30%, 23% and 46.7% for the control, diesel exhaust and particle-free exhaust groups, respectively. The difference in total lung tumor rate between the control and particle-free exhaust groups was of borderline statistical significance ($p = 0.053$). No statistical

analysis was presented for adenocarcinoma incidence alone as opposed to total lung tumor incidence.

After 24 months of exposure, the mortality rates in the C57BL/6N mice were 55%, 58% and 67% in the control, particle-free exhaust and diesel exhaust groups, respectively. The 50% mortality rate occurred after 25 months in the diesel exhaust group and at 27 months in the other two groups. The authors did not indicate if the difference in mortality between the controls and the diesel exhaust-exposed animals was significant. Mean body weights were not significantly altered by treatment compared to controls. Mean lung wet weights of the diesel exhaust-exposed were doubled after 6 months of exposure when compared to controls. Mean lung particle burdens after 3, 6, 12, 18 and 21 months of exposure were 0.8, 2.3, 3.5, 4.3 and 5.5 mg/lung for the diesel exhaust exposure group. The mean lung particle load after 21 months of exposure (expressed as mg particles/g clean air control lung) was 31.6 mg. The total (benign and malignant) lung tumor rates for diesel exhaust and particle-free exhaust exposed animals (8.5% and 3.5%) were not significantly increased compared to controls (5.1%).

Mauderly *et al.*, 1996

This study presents results from a diesel exhaust carcinogenicity bioassay using CD-1 mice performed at the same time as a previously reported diesel exhaust bioassay using Fischer 344 rats (Mauderly *et al*., 1987). Male and female CD-1 mice (17 weeks of age) were exposed to diesel exhaust (particle content 0.35, 3.5 or 7.1 mg/m^3) for 7 hours/day, 5 days/week for 24 months. A clean air control group was also included. The number of mice evaluated for tumors ranged from 59 to 82 males and from 88 to 104 females per treatment group. Animals were entered into the treatment groups at three times (12-13 months apart), and exposed and treated identically. Diesel exhaust was generated by 1980 model 5.7-liter Oldsmobile V-8 engines operated continuously on the U.S. Federal Test Procedure urban certification cycle.

The median life spans of males in the 0.35 (490 days) and 3.5 mg/m^3 (450 days) exposure groups were significantly reduced compared to controls (550 days). However, the median life spans of the male 7.1 mg/m^3 exposure group (561 days) and all of the female exposure groups were not significantly different from controls. Mean lung particle burdens after 18 months of exposure were 0.2, 3.7 and 5.6 mg/lung for the 0.35, 3.5, and 7.1 mg/m^3 exposure groups, respectively.. Alveolar/bronchiolar adenomas and adenocarcinomas were observed in the lungs of both diesel exhaust-exposed and control mice. However, lung tumor incidences in the exposed groups were not significantly different from controls as determined by logistic regression analysis (see Table 6.1.b).

6.1.1.2 RATS:

In rats the results of eleven cancer bioassays of inhalation of diesel exhaust alone are presented in Table 6.1c. None of the four studies with either exposure periods of less than 7 hours/day, 5 days/week for 24 months or particulate exposure concentrations of less than 2.2 mg/m^3 (Karagianes *et al*., 1981; White *et al*., 1983; Lewis *et al*., 1986, 1989; Takemoto *et al*., 1986) gave positive results for carcinogenesis of diesel exhaust. These studies are not discussed in

detail here but are discussed under non-cancer effects; the studies by Karagianes *et al*., (1981), White *et al*., (1983) and Takemoto *et al*., (1986) were also reviewed by IARC (1990). The seven studies which presented positive results (Brightwell *et al*., 1986, 1989; Heinrich *et al*., 1986; Ishinishi *et al*., 1986a; Iwai 1986; Mauderly *et al.*, 1987a; Heinrich *et al*., 1995; Nikula *et al*., 1995) are discussed below.

Brightwell *et al*., 1986, 1989:

This chronic inhalation study examined the comparative toxicity and carcinogenicity of four types of engine exhaust emissions. The emissions used were those from (1) a gasoline engine; (2) a gasoline engine fitted with a 3-way catalytic converter; (3) a diesel engine; and (4) a diesel engine with particle filtration. The exhaust emissions used in this study were generated by two Renault R18 1.6-liter gasoline engines and a VW Rabbit 1.5-liter diesel engine. All engines were run according to the US-72 (FTP) driving cycle. Normal standard diesel fuel (European quality) and US quality lead-free gasoline (Shell, Switzerland) were used for the engines.
The study compared ten experimental groups of Fischer 344 rats. Three concentration levels were used for the unfiltered diesel exposed animals (particle concentrations for low - 0.7 mg/m^3, medium - 2.2 mg/m^3, and high - 6.6 mg/m^3), two concentration levels for the filtered diesel exposed groups (same as unfiltered medium and high dose except particles removed), and two concentration levels for each of the gasoline emission types (medium, 1.2% exhaust (v/v) and high, 3.5% exhaust (v/v)). Control animals were exposed to clean air. Each experimental group (except for controls) contained 144 rats. The control group contained 288 rats. Each group contained an equal number of males and females.

Exposures to the test or control atmospheres were carried out overnight with five 16-hour exposure periods per week. This schedule resulted in exposure during the active phase of the animal's diurnal cycle. Exposures were continued for 2 years. Rats surviving at the end of exposure were maintained for a further 6 months without exposure to exhaust emissions.

Interim sacrifices of rats were carried out at 6, 12, 18, and 24 months. Body weights were determined at 3-week intervals. All animals that died or were sacrificed were subject to a full necropsy, with all major organs being preserved in 10% buffered formalin. Histopathological examination was carried out on the respiratory tract (nasal passages, larynx, trachea and lungs) in all high dose and control animals. Histological examinations were also carried out on suspected tumors in all animals, regardless of their experimental treatment.

Apart from the unfiltered diesel exposure groups, the concentration of exhaust emission particles in all chambers was below the limit of detection (< 0.21 mg/m^3). In the unfiltered diesel exhaust chambers the mean particle concentrations were 0.7, 2.2 and 6.6 mg/m^3 for the low-, medium- and high-concentration chambers, respectively. The median diameters of particles were 0.04, 0.08 and 0.14 μm for the gasoline, gasoline plus converter, and unfiltered diesel exhaust, respectively. The number of particles in the filtered diesel exhaust chamber was too low for size distribution measurements to be made.

Statistically significant differences in body weight between emission-exposed and control animals were observed. A clear treatment-related reduction in growth rate in the gasoline high-dose group and in unfiltered diesel groups was observed compared with controls. No consistent differences were observed in any other treatment groups.

Changes in organ weights were also reported. The most marked and consistent change was an increase of lung weight in male and female rats exposed to unfiltered diesel exhaust, compared with controls. This was statistically significant only in the medium and high dose groups. There was a steady increase in the magnitude of the changes with increasing exposure time.

No increase in the overall incidence of respiratory tract tumors was observed in the gasoline, gasoline catalyst or filtered diesel exposed groups. Compared to the control group, the medium and high dose unfiltered diesel groups (2.2 and 6.6 mg/m^3) showed an increase in respiratory tumor incidence in rats. The incidence of lung tumors in females was considerably higher than in males. The overall total number of rats with primary lung tumors was as follows: control - males 2/134 (1.5%), females 1/126 (0.8%); 0.7 mg/m^3 - males 1/72 (1.4%), females 0/71 (0%); 2.2 mg/m^3 - males 3/72 (4.2%), females 11/72 (15.3%); and 6.6 mg/m^3 - males 16/71 (22.5%), females 39/72 (54.2%).

The majority of lung tumors appeared during the 6-month observation period which followed 24 months of exposure. The highest incidence of tumors was seen in female rats dying after the end of exposure (25 - 30 months) in the unfiltered diesel high-dose group. These animals showed a primary lung tumor incidence of 96% (24 out of 25 animals), with 79% (19) of the tumors diagnosed as malignant. The highest incidence of tumors in males was also seen after 24 months, when the tumor incidence was 44% (12/27), with 83% (10/12) of the tumors diagnosed as malignant.

Many of the rats exposed to unfiltered diesel exhaust exhibited multiple lung tumors. For example, 39 of the 72 female rats in the high dose group exhibited lung tumors, but a total of 75 primary lung tumors was identified in these 39 animals. Within any one animal the tumors were often of markedly different histological types. In both males and females subject to the high exposure of unfiltered diesel exhaust, the following types and numbers of primary lung tumors were identified: 40 adenomas; 35 squamous cell carcinoma; l9 adenocarcinomas; 9 mixed adenoma/adenocarcinoma/squamous cell carcinoma; and l mesothelioma.

Heinrich *et al*., 1986a:

Female Wistar SPF (specific pathogen free) rats were exposed for 19 h/d, 5 d/wk for 140 weeks starting at 8 to 10 weeks of age. The animals in groups of 96 were exposed to clean air, unfiltered diesel exhaust (4 mg/m^3), filtered diesel exhaust diluted by the same amount (17-to-1), and coke oven flue gas (4-7 mg/m^3). The diesel exhaust, source and exposure set-up were identical to that reported for the study conducted by the same authors in mice (see Section 6.1.1.1). A variety of physiological, biochemical and cytological data were collected at 1 and 2 years of exposure. Full histological evaluations were conducted at scheduled or moribund sacrifices.

The body weights of the rats exposed to the filtered exhaust did not differ significantly from controls. The body weights of rats exposed to unfiltered diesel exhaust fell significantly below control values after approximately 480 days. After this time the weight gain of the exposed rats was consistently retarded compared with the controls. The median experimental lifetime of the animals, i.e. the time period of exposures after which 50% of the animals of a group were still alive, was not significantly influenced by the exposures.

After one year of exposure to unfiltered diesel exhaust, the wet and dry weights of the lungs of rats significantly increased in comparison to the controls. After two years the difference amounted to 3 to 4 times higher in unfiltered exhaust-exposed animals in comparison to the control.

After 140 weeks of exposure no lung tumors were reported in the clean air or in the filtered exhaust-exposed animals. After the exposures to unfiltered diesel exhaust, significant morphological changes occurred. In this exposure group 15 out of 95 (approximately 16%) rat lungs examined exhibited a total of 17 tumors. They were diagnosed as 8 bronchial-alveolar adenomas and 9 squamous cell tumors (8 benign cysts, 1 carcinoma). The inclusion of benign cysts as tumors is not done in most risk analyses (including this one); generally a tumor is defined for risk assessment as a lesion that either is malignant or may be expected to become malignant. In addition, out of 95 rats, 94 exhibited hyperplasia and 62 exhibited metaplasia in their lungs. There was additional evidence of severe inflammatory changes, including thickened alveolar septa and foci of macrophages. The upper respiratory tract showed no exposure-related changes. These results suggest that in the rat lung the inhaled particles were involved in the induction of the lung tumors found. This conclusion differs from that reported by the same authors in mice (see Section 6.1.1.1), where increased malignancies were found in both the filtered and unfiltered exhaust.

In comparison, rats exposed to the coke oven flue gas (Heinrich *et al*., 1986b), which has nearly 1000-fold more BaP than the diesel exhaust, exhibited less severe non-neoplastic changes than the rats exposed to unfiltered diesel exhaust. However, despite the lesser incidence of non-neoplastic changes, 21 of 116 rats (18.1%) exhibited 22 lung tumors. These tumors were diagnosed as 1 bronchio-alveolar adenoma, 1 bronchio-alveolar carcinoma, and 20 squamous cell carcinomas, a very significant increase above the control incidence of zero.

Ishinishi *et al*., 1986, 1988:

This study evaluated the carcinogenicity of the exhaust of a light-duty (LD) and a heavy duty (HD) diesel engine. Groups of 64 male and 59 female Fischer 344/Jcl rats (SPF) were exposed to whole diesel exhaust diluted to varying degrees with clean air for 16 hr/day, 6 days/wk, for up to 30 months from age 5 weeks. Sacrifices of (usually) 14 males and nine females occurred at six-month intervals. In the LD test, the diesel exhaust particulate concentrations were 0.1, 0.4, 1, and 2 mg/m^3. In the HD test the 4 exhaust particulate matter concentrations were 0.4, 1, 2, and 4 mg/m^3, also obtained by dilution. Both studies had controls exposed to clean air. The diesel fuel was JIS No. 1 or No. 2 light oil.

The authors reported only minor differences among survival rates and body weight in the different exposure groups. Black discoloration of lungs in the exposed animals increased with concentration and time of exposure. Marked hyperplasia of the type II epithelial cells occurred in the higher concentration groups after 18 months. With exposure, adenomatous hyperplasia increased to 99 lesions in 247 animals at the highest LD exposure and 39 lesions in 247 animals at the highest HD exposure. At the distal ends of the respiratory bronchioles in regions of carbon deposition, the bronchiolar epithelium usually extended continuously toward the alveolar ducts.

Incidences of neoplasms in the LD tests were 4/123, 3/123, 1/125, 5/123, 3/124 for the 0, 0.1, 0.4, 1.0, and 2.0 mg/m^3 exposure groups respectively. These results do not represent a statistically significant trend with increasing exposure. Incidences of neoplasms in the HD tests were 1/123, 1/123, 0/125, 4/123, 8/124 for the 0, 0.4, 1.0, 2.0, and 4.0 mg/m^3 exposure groups, respectively. The incidence at the highest exposure in the HD test is the only one of these incidences that, by itself, differs significantly ($p<0.02$ by the Fischer exact test) from the relevant control value.

In both tests together, there were four adenomas, 16 adenocarcinomas, 5 adenosquamous carcinomas and 5 squamous carcinomas. Regions of adenomatous hyperplasia were often noted in the vicinity of the cancer foci.

Iwai *et al.*, 1986:

Female Fischer 344 (SPF) rats in groups of 24 were exposed unfiltered diesel exhaust containing 4.9 mg/m^3 particulate matter, filtered diesel exhaust, or clean air for 8 hr/d, 7d/wk and continued for 24 months. A 2.4 liter diesel truck engine generated the exhaust. Animals were necropsied at 3, 6, 12, and 24 months of exposure. Some of the rats exposed for 12 or 24 months were thereafter kept in clean air for three or six months, respectively, and then necropsied.

The rats in the unfiltered diesel exhaust group showed lower body weights compared with those in the filtered exhaust group and the control group. The ratio of lung weight to body weight increased after 12 months exposure to unfiltered exhaust to over twice that of the control group, and approximately three times after 24 months of exposure. In the rats exposed 24 months to filtered exhaust, the ratio of lung weight to body weight increased only slightly compared to controls.

No lung tumors were observed in the 15 rats that died prior to the main sacrifice at the end of the two-year exposure period. In the 15 rats exposed to clean air for 24 months no lung tumors were found, but in seven 30-month control rats one adenoma was observed. The resulting incidence of lung tumors in the control rats surviving two years or more was 4.5% (1/22). In the rats of the filtered-exhaust group no lung tumors were found. In the unfiltered-exhaust group 4 out of 14 rats had lung tumors at the end of the two year exposure, 2 of them being diagnosed as malignant. In the group maintained in clean air after two years exposure to unfiltered exhaust, 4/5 rats had lung tumors, 3 of which were malignant. Therefore, among the 19 rats exposed to unfiltered exhaust for two years, a total of eight (42.1%) bore lung tumors, five (26.3%) of which were malignant. The incidence showed a significant difference ($p<0.01$) between the control and

the unfiltered exhaust groups. The histological types and number of tumors were: 3 adenomas, 1 adenocarcinoma, 2 adenosquamous carcinomas, 1 squamous carcinoma, and 1 large cell carcinoma.

In the unfiltered (25%) as well as the filtered (37.3%) exhaust groups, splenic malignant lymphomas were found to be elevated ($p<0.05$) compared to controls (8.3%). This seems to be the first report of malignancies of the spleen due to inhaled diesel exhaust. The authors reported that the large-scale historical occurrence of lymphoma/leukemia in Fischer 344 rats ranged from 8.3% to 18.8%. In this experiment the significant differences in the rate of malignant lymphoma of the spleen of the two exposed groups, frequently complicated by leukemia, was attributed by the authors to the effect of gas phase, accelerating an occurrence of the disease in Fischer 344 rats.

The tumor incidences in organs other than the lung or spleen were: 8.2%, 25% and 29.1% in the control, filtered and unfiltered diesel exhaust exposure groups, respectively. The statistical significance of these data was not reported. The number of animals exhibiting multiple tumors (i.e. greater than 1 tumor per animal) was greater in exhaust exposed groups than in controls.

Mauderly *et al*., 1987a:

Male and female SPF F344/Crl rats (age 17 weeks) were exposed to diesel engine exhaust at particulate concentrations of 0.35, 3.5 and 7.0 mg/m^3 or to clean air for 7 h/d, 5 d/wk for up to 30 months. The MMAD of the diesel exhaust particulate was 0.25 µm. The exhaust was generated by a 1980 Model 5.7L Oldsmobile V-8 engine using D-2 diesel control fuel meeting US EPA certification standards.

A subgroup of each exposure group was killed at 6-month intervals for evaluations such as lung burdens of soot, histopathology, and immune responses. Of the serially sacrificed rats, only those designated for histopathology were examined for lung tumors. The remaining rats, those which either died spontaneously, were euthanized when found moribund or survived to 30 months, were also examined histologically. Of a total of 364-367 rats entered into each treatment group, 221-230 were examined for lung tumors.

Diesel exhaust exposure did not cause overt signs of toxicity. When the data from both sexes were combined, the median survival time of the high-level exposure group was slightly, but not significantly shorter than the control group. Exhaust particulate matter progressively accumulated in lungs of rats at all exposure levels. The increase in lung burden of particulate matter was significant for all groups and was much larger in the two highest exposure groups than at the lowest exposure concentration. Lung disease was evident at the middle and high-level exposure groups, and paralleled the soot accumulation. A chronic, active inflammation accompanied focal accumulation of soot in alveolar macrophages. Epithelial cells were altered, and epithelial hyperplasia consisted of bronchiolar cell types intruding into the alveoli. Metaplasia occurred adjacent to fibrotic foci. The lesions were focal and much of the lung parenchyma appeared normal.

The authors reported an overall tumor incidence for each exposure group. They combined the data from the serially sacrificed animals with the data from those animals which died, were euthanized or were terminated at the end of the study. The overall prevalence of lung tumors was significantly ($p<0.05$) increased at the high (29/227, 12.8%) and medium (8/221, 3.6%) dose levels above the control prevalence (2/230, 0.9%). The tumor incidence in the low dose group was similar to controls (3/223, 1.3%).

Four tumor types, all of epithelial origin, were observed: adenoma, adenocarcinoma, squamous cyst, and squamous cell carcinoma. The tumors at the high level were both malignant tumors and squamous cysts. As explained in reviewing Heinrich *et al*. (1986a) above, the definition of tumor in a risk assessment excludes those lesions not expected to progress to malignancy. Eleven of the 33 lesions reported as tumors were (benign) squamous cysts in the highest dose group. The tumors at the medium level were primarily adenomas, although 2 were designated squamous cysts. The tumors described were incidental findings at death or termination. None of the tumors were found to have metastasized to pulmonary lymph nodes or to other organs.

The vast majority of tumors was detected from 24-months to the end of the study at 30 months. The time of tumor observation is particularly noteworthy in view of the 24-month observation time commonly used for chronic carcinogenesis studies in rats. Of all rats observed to have tumors, only 19% were identified at or before the 24-month termination.

In interpreting this study, it is appropriate to keep in mind that at the higher exposure levels rats accumulated lung burdens of particulate matter greater than those which would be predicted from results at the low exposure level. It has been shown (Section 3) that this effect is associated with a slowing of the clearance of particles from the lung after prolonged exposure of rats to diesel exhaust. This finding suggests that caution should be exercised in extrapolating the lung tumor prevalence among rats exposed to high levels of exhaust to potential carcinogenicity among people exposed to lower levels.

The authors pointed out that the finding of exhaust-induced tumors is consistent with an initiation-promotion mechanism of chemical carcinogenesis. DNA extracted from lungs of high level rats in the present study after 30 months of exposure was shown to have an increased number of adducts in comparison to DNA extracted from lungs of controls (Wong *et al*., 1986) (see Section 5). This finding suggests that interaction of reactive metabolites of soot-associated organic compounds with lung cell DNA provided an initiating event. The progressive accumulation of diesel particulate may have provided a continuous source of mutagenic compounds. The chronic inflammation and epithelial cell proliferation could have acted as promoting influences.

The authors also pointed out that the squamous tumors might have been a generalized response of the lung to the presence of large quantities of relatively insoluble foreign material. The squamous tumors in the present study were always associated with areas of soot deposition, epithelial cell alterations, and fibrosis, while the other tumor types were not always associated with these lesions. The squamous tumors, therefore, may very likely have progressed from the focal squamous metaplasia.

Heinrich *et al*., 1995:

Female Wistar rats were exposed to diesel exhaust (0.8, 2.5 or 7 mg/m^3), carbon black (7.4 mg/m^3 for 4 months, followed by 12.2 mg/m^3 for 20 months), or titanium dioxide (TiO_2) (7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 16 months) for 24 months, followed by clean air for 6 months. Appropriate clean air controls were included. The number of animals/group examined for tumors were 220 for the clean air controls, 200 for the 2.5 and 0.8 mg/m^3 diesel exhaust exposure groups and 100 for the 7 mg/m^3 diesel exhaust, carbon black and TiO_2 exposure groups. Diesel exhaust was generated as described in Section 6.1.1.1 (results in mice). All animals were 7 weeks of age at the start of exposure, and were exposed for 18 hours/day, 5 days/week. The carbon black and TiO_2 exposure concentrations were varied to maintain a lung particle load comparable to the 7 mg/m^3 diesel exhaust exposure group as determined from data obtained at interim sacrifices.

The mortality rates after 24 months of exposure were 42% (controls), 45% (0.8 mg/m^3 diesel exhaust), 52% (2.5 mg/m^3 diesel exhaust), 47% (7 mg/m^3 diesel exhaust), 56% (carbon black) and 60% (TiO_2). After 130 weeks (exposure time followed by clean air) the mortality rates were 85% (controls), 86% (0.8 mg/m^3 diesel exhaust), 89% (2.5 mg/m^3 diesel exhaust), 82% (7 mg/m^3 diesel exhaust), 92% (carbon black) and 90% (TiO_2). Mean body weight was significantly reduced compared to controls starting at day 440, 200, 300 and 400 for the 2.5 mg/m^3 diesel exhaust, 7 mg/m^3 diesel exhaust, carbon black and TiO_2 exposure groups, respectively. Mean body weights for those groups were also significantly lower than those of controls at the end of the 24 month exposure.

Lung wet weights were determined at 3, 6, 12, 18, 22 and 24 months of exposure. Mean lung wet weights were not increased in the 0.8 mg/m^3 diesel exhaust compared to controls, and were not increased in the 2.5 mg/m^3 diesel exhaust until 22 months of exposure. Mean lung wet weights of the 7 mg/m^3 diesel exhaust exposure group increased consistently throughout the 24 month exposure period, and increased in the carbon black and TiO_2 exposure group up to 18 months. Those weights then either plateaued or slightly decreased for the remainder of the exposure period. Lung particle (including lung-associated lymph nodes) burden increased consistently through the exposure period for all treatment groups. The lung particle burden for the 7 mg/m^3 diesel exhaust, carbon black and TiO_2 exposure groups was similar for the first 12 months of exposure. Lung particle burden increased to a much greater degree during the second 12 months of exposure for the 7 mg/m^3 diesel exhaust group (80% increase) than for the carbon black and TiO_2 exposure groups (13% and 15% increases, respectively).

Lung tumors were not observed in the 7 mg/m^3 diesel exhaust, carbon black and TiO_2 exposure groups at 6 and 12 months of exposure (interim sacrifice of approximately 20 animals/group). After an experimental period of 30 months (24 months of exposure followed by 6 months of clean air), lung tumor (benign and malignant, including benign squamous-cell tumors) incidences were significantly increased in the 2.5 mg/m^3 diesel exhaust (5.5%), 7 mg/m^3 diesel exhaust (22%), carbon black (39%) and TiO_2 (32%) exposure groups. No lung tumors were observed in the 0.8 mg/m^3 diesel exhaust group, and one lung tumor (an adenocarcinoma) was found in the control group. Lung tumor incidences between the various exposure groups were compared

using cumulative particle exposures (particle concentration multiplied by exposure time), since a comparison using particle concentration was not possible due to the variable exposure concentrations used with the carbon black and TiO_2 groups. The authors stated that a comparison using cumulative particle exposures indicated that for all particle types combined, total lung tumor rate increased with increasing cumulative particle exposure. The total tumor incidence of the carbon black group (39%) was significantly greater than the 7 mg/m^3 diesel exhaust group (22%) after taking survival into account; the difference between the TiO_2 (32%) and 7 mg/m^3 diesel exhaust groups was not significant.

Nikula *et al*., 1995:

Male and female Fischer 344/N rats (7 to 9 weeks of age) were exposed to diesel exhaust or carbon black 16 hours/day, 5 days/week for 23 months. Target particle concentrations of diesel exhaust and carbon black were 2.5 and 6.5 mg/m^3. Diesel exhaust was generated using two 1988 Model LH6 General Motors 6.2-liter V-8 engines operated on the Federal Test Procedure urban certification cycle. Particle size was bimodal for both diesel exhaust and carbon black; the mass median aerodynamic diameter (MMAD) of the large-size mode was 2.0 µm and 1.95 µm for diesel exhaust and carbon black, respectively. The MMAD for the small-size mode was 0.1 µm for both diesel exhaust and carbon black. Approximately 23% and 67% by mass of the diesel exhaust and carbon black particles, respectively, were in the large-size mode. Treatment groups ranged in size from 114-118 and 114-116 animals/group for males and females, respectively. Interim sacrifices were conducted at 3, 6, 12, 18 and 23 months of exposure using 6 animals/group (3 male, 3 female).

Mortality in males was increased compared to corresponding controls in both carbon black exposure groups after approximately 500 days, and in the 6.5 mg/m^3 diesel exhaust group after about 600 days. At 23 months, percent cumulative mortality in males was 86% for controls, 86% and 94% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and 96% and 99% for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively. Percent cumulative mortality in females was 64% for controls, 69% and 73% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and 60% and 74% for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively. Body weights were reduced by all treatments except for 2.5 mg/m^3 diesel exhaust exposure.

Significantly increased lung wet weights compared to controls were observed in males at 18 and 6 months for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and at 12 months for both carbon black exposure groups. Similar increases in females were noted at 12 and 6 months for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and at 6 and 3 months for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively. The increase in lung particle burden due to diesel exhaust and carbon black exposure tended to accelerate in both genders after 12 months of exposure; diesel exhaust soot accumulated more rapidly than carbon black particles. Mean lung particle burdens (mg/lung) at 23 months for males were 45.1 and 90.1 mg for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and 24.7 and 74 mg for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively. Mean lung particle burdens for females were 36.7 and 80.7 mg for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively, and 17.3 and 36.9 mg for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively.

The authors stated that all primary lung neoplasms appeared to be of parenchymal origin; none appeared to originate from the conducting airways. Lung tumors tended to appear late in the exposure period (> 600 days exposure) in both the diesel exhaust and carbon black groups, and occurred earlier in the high concentration group compared to the low exposure group for both test materials. Both exposure concentrations of diesel exhaust and carbon black caused increased total (benign and malignant) lung tumor incidence in female rats. Incidence rates were 8/107 (7.5%) and 28/105 (26.7%) for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively, and 8/105 (7.6%) and 29/106 (27.4%) for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively. The female control incidence rate was 0/105 (0%). Males were less susceptible to both diesel exhaust-induced and carbon black-induced lung tumors than were females. Total tumor incidence rates were 2/106 (1.9%) and 4/106 (3.8%) for the 2.5 and 6.5 mg/m^3 carbon black groups, respectively, and 5/105 (4.8%) and 9/106 (8.5%) for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively. The male control incidence rate was 3/109 (2.8%). The authors state that logistic regression modeling did not show significant differences between the tumor responses to diesel exhaust and carbon black for either gender. However, they also noted that slope estimate errors in the logistic regression models were large; the errors were particularly large for the males, because of their much shorter lifespan compared to the females. Lung tumor incidence in the females increased rapidly towards the end of their lifespan, when most of the males had died of other causes. Also, the lack of lung tumors in the control females may have increased the estimated slopes for the treatment group females. The authors note that these factors make it difficult to determine if there were true gender differences in neoplastic incidence or differences in the responses of males to diesel exhaust and carbon black.

6.1.1.3 MONKEYS

Lewis *et al*., 1986, 1989:

Male cynomolgus monkeys (Macaca fascicularis) were exposed to diesel exhaust (2 mg/m^3), respirable coal dust (2 mg/m^3; particle size < 7 µm), or diesel exhaust (1 mg/m^3) combined with coal dust (1 mg/m^3) for 7 hours/day, 5 days/week for 24 months. Appropriate clean air controls were included. Exposure groups consisted of 15 animals. Animals weighed 4-5 kg at acquisition; the animals were then quarantined for 3 months prior to the start of the study. Diesel exhaust was generated by a Caterpillar Model 3304 diesel engine on an operation cycle which was designed to simulate the load-haul-dump operation of diesel-powered trams used in coal mining. The mass median diameter of the diesel exhaust particulate matter was determined to be either 0.23 or 0.36 µm using either an aerosol size analyzer or scanning electron microscopy techniques, respectively.

One animal in the coal dust group died of diabetes mellitus prior to the end of the exposure period; all other animals survived to necropsy. Body weights were not affected by either diesel exhaust or coal dust exposure, separately or combined. All organs and tissues from the exposed animals were examined grossly at necropsy. The authors also stated that 36 different tissue and organs were examined histopathologically; the specific tissues and organs examined were not listed. No significant treatment-related difference in tumor incidence was noted for any of the exposure groups. Specific tumor incidence data were not reported. Also, the small number of

animals/exposure group (15) and the considerably less than lifetime exposure (2 years exposure compared to a 16-30 year lifetime) suggest that the sensitivity of this study to any increased tumor incidence due to diesel exhaust exposure was low.

Lung sections from the exposed monkeys and male F344 rats from the Lewis *et al*. (1986; 1989) studies also exposed to diesel exhaust were examined histopathologically by Nikula *et al*. (1997). The rats (8 - 10 weeks old at exposure initiation) were exposed in whole body chambers 7 hours/day, 5 days/week for up to 24 months to diesel exhaust, respirable coal dust, or diesel exhaust combined with coal dust at the concentrations listed above. In all groups, the relative volume densities and volume percentages of retained particulate matter was greater in monkeys than in rats. However, there was no significant difference in the control-adjusted relative amount of retained particulate material between diesel exhaust-exposed monkeys and rats. Diesel exhaust-exposed monkeys retained slightly more particulate matter in the lung interstitium than in the alveoli/alveolar duct lumens; diesel exhaust-exposed rats retained considerably more particulate matter in the alveoli/alveolar duct lumens than in the lung interstitium. A scale ranging from 1 to 5 was used to describe severity scoring of inflammation indices; the descriptors were 1 (slight), 2 (minimal), 3 (mild), 4 (moderate) and 5 (marked). Both rats and monkeys demonstrated a significant increase in the incidence of alveolar macrophage hyperplasia in the diesel exhaust-exposed animals compared to controls. Mean severity scores for rats and monkeys were 1.7 and 1.2, respectively. The incidence of diesel exhaust-induced alveolar epithelial hyperplasia, particle-associated inflammation and septal fibrotic reaction was significantly increased in rats but not monkeys. However, the severity scores for alveolar epithelial hyperplasia, particle-associated inflammation and septal fibrotic reaction (1.7, 1.1 and 1.1, respectively) in diesel exhaust-exposed rats were not large, and the scores for the monkeys that did demonstrate alveolar epithelial hyperplasia and particle-associated inflammation (1.5 and 1.0, respectively) were similar to those for diesel exhaust-exposed rats (1.7 and 1.1, respectively). As noted above, the sample sizes used (15 for control and diesel exhaust-exposed rats and diesel exhaust-exposed monkeys; 14 for control monkeys) were relatively small, indicating that the power of this study to detect any increased incidence of inflammation indices due to diesel exhaust exposure was low.

6.1.2 INHALATION OF DIESEL EXHAUST WITH CO-ADMINISTRATION OF KNOWN CARCINOGENS

An important question is whether diesel engine exhaust acts synergistically with other agents known to be carcinogenic. Several investigators have examined the effects of co-administration of known carcinogens with inhalation of diesel exhaust in hamsters, mice and rats. These studies are summarized in Table 6.2.a-c. The carcinogens examined thus far include: urethane, BaP (benzo[a]pyrene); BHT (butylated hydroxytoluene); DBahA (dibenzo[ah]anthracene); DIPN (diisopropanol-nitrosamine); DEN (diethylnitrosamine) and DPN (dipentylnitrosamine).

There is no consistent evidence of synergism between inhaled diesel exhaust and injected DEN or BaP in hamsters, under the experimental conditions used (Heinrich *et al*., 1982; 1986a; 1986b; Brightwell *et al*., 1989). See Table 6.2.a.

The effects of diesel exhaust inhalation on the carcinogenic activity of urethane, BHT (butylated hydroxytoluene), BaP, and DBahA (dibenzo[ah]anthracene) have been examined by Pepelko and Peirano (1983) and Heinrich *et al*., (1986a) in mice. See Table 6.2.b.

In two pulmonary adenoma assays, Pepelko and Peirano (1983) exposed male and female Strong A mice for 8 h/d, 7 d/wk from six weeks to nine months of age to clean air or to unfiltered diesel exhaust. At the start of exposure, half of each group also received a single intraperitoneal (i.p.) injection of urethane.

In the first assay, mice were exposed to diesel exhaust containing 6 mg/m^3 particulate matter and half the group was injected with 1 mg urethane. Each of the four groups (control, exposure only, injection only, exposure and injection) had 60 females. In this assay the diesel exhaust was apparently carcinogenic, and the injections of urethane had no apparent effect. Without the injection, the number of mice with tumors increased significantly, from 4 out of 58 for clean air exposure to 14 out of 56, for exposure to 6 mg/m^3 of diesel exhaust. With the 1 mg injection, the increase in the number of mice with tumors, from 9 out of 52 for clean air exposure to 22 out of 59 for diesel exhaust exposure was also statistically significant ($p < 0.02$).

In the second assay, mice were exposed to diesel exhaust containing 12 mg/m^3 particulate matter, and injected with 5 mg urethane. Each of the four experimental groups had 45 males and 45 females. In this assay, the diesel exhaust appeared to reduce the number of female mice with tumors, relative to clean air controls from 11/43 to 4/43. In addition, urethane induced tumors were significantly reduced in male and female mice exposed to diesel exhaust relative to clean air exposed controls.

Pepelko and Peirano (1983) also investigated the effect of diesel exhaust on butylated hydroxytoluene (BHT) and urethane carcinogenic activity in Sencar mice. The effects were examined in a two-generation study. The parent generation was exposed continuously to clean air or 6 mg/m^3 diesel exhaust from weaning age to sexual maturity and then mated. Exposure of the dams was continued through pregnancy, parturition, and weaning of offspring. The concentration was increased to 12 mg/m^3 when the mean age of the offspring was 12 weeks and continued until termination of the study at 15 months of age. The offspring in each clean air or exposure group were divided and assigned to three treatment groups of 260 each, 130 males and 130 females: 1) serial injection of BHT beginning at seven weeks of age and lasting one year to test for tumor initiating activity (doses: 300 mg/kg the first week; 83 mg/kg the second week; 150 mg/kg thereafter), 2) a single injection of 1 mg urethane at six weeks of age to test for tumor promoting activity, 3) no injection.

In females of the uninjected group, diesel exhaust significantly increased the incidence of adenomas, from 6.3 to 16.3% ($p=0.02$), and the total lung tumors, from 7.2 to 16.3% ($p=0.05$). The increase was also statistically significant with sexes combined. In females injected with BHT, diesel exhaust exposure significantly decreased the incidence of adenomas, from 16.7 to 3.9% ($p=0.01$), and of all lung tumors from 18.l% to 6.5% ($p=0.03$). This did not occur in the male mice, however. No significant differences were detected between incidences in the diesel exhaust/urethane and the clean air/urethane groups of either sex.

Heinrich *et al*. (1986a) investigated the combined effects of diesel exhaust and BaP or DBahA. Female NMRI mice (8-10 weeks old) were exposed for 19 h/d, 5 d/wk for 120 wks to clean air, 4 mg/m^3 unfiltered, or filtered diesel exhaust. The 2 PAHs were instilled intratracheally into each of the three inhalation exposure groups of 64 mice: 50 or 100 µg BaP, once per week for 20 or 10 weeks, respectively; or 50 µg DBahA once per week for 10 weeks. In addition, groups of 96 mice received one subcutaneous injection of 10 or 5 µg DBahA near birth, and they were examined at six months.

With clean air the lung tumor incidence following instillation of 20 x 50 µg BaP was 71%, and with exposure to unfiltered exhaust, the incidence was reduced to 41%. This significant change was due to a decreased incidence of adenocarcinomas, with no change in adenoma incidence. Although the authors did not present the data from the 10 x 100 µg BaP treatment groups, they stated that the change in tumor incidences observed with 20 x 50 µg BaP instillations was not reproduced in the 10 x 100 µg BaP groups even though the total dose of BaP was the same. The authors also did not report the data from the DBahA groups; however, they did state that no significant differences between clean air/DBahA and diesel exhaust/DBahA groups were observed. Of the mice injected with the lower dose of DBahA near birth, 46% of the clean air group developed tumors, and the exhaust exposed groups did not differ significantly from that. Of the mice injected with the higher dose, 81% of the clean air group developed tumors, and 63% of the group exposed to whole diesel exhaust developed tumors, a statistically significant difference. These data indicate that breathing diesel exhaust did not significantly increase the number of tumors produced with administration of BaP or DBahA.

To evaluate the effect of diesel exhaust exposure on the carcinogenic activity of dipentylnitrosamine (DPN), Heinrich *et al*. (1986a) exposed female Wistar SPF (specific pathogen free) rats for 19 h/d, 5 d/wk for 140 weeks starting at 8 to 10 weeks of age. The animals were exposed to clean air, filtered diesel exhaust, and unfiltered diesel exhaust (4 mg/m^3 particles). A subgroup of 48 animals from each exposure group was given subcutaneous injections of 0 or 250 mg or 500 mg/kg DPN for each of the first 25 weeks of inhalation exposure. The diesel exhaust source and exposure set-up were identical to that reported for the study conducted by the same authors in mice (see above).

The groups of animals breathing clean air and injected with DPN exhibited high incidence of tumors in the lungs (85% and 94% for the lower and higher DPN levels, respectively). Most of the malignant tumors were adenocarcinomas and the remainder were squamous cell carcinomas. DPN also induced tumors in the upper respiratory tract, primarily in the nasal cavities. At the high DPN dose, tumors were also observed in the larynx and trachea. Compared to clean air, exposure to filtered or unfiltered diesel exhaust resulted in significant decreases in the benign tumors in the upper respiratory tract for both DPN dose levels. However, compared to clean air, exposure to unfiltered diesel exhaust significantly increased the occurrence of squamous cell carcinomas in the lungs at both DPN dose levels. This increase was not observed in DPN animals exposed to filtered diesel exhaust. DPN-induced increases in liver and kidney tumor rates were not influenced by diesel exhaust exposure.

Takemoto *et al*. (1986) evaluated the combined effect of di-isopropanol nitrosamine (DIPN) and diesel exhaust in female Fischer 344/Jcl rats. Exposures to clean air or 2-4 mg/m^3 unfiltered diesel started at 5 weeks of age for 4 h/d, 4 d/wk for up to 24 months. One month after starting inhalation exposures one clean-air group and one exhaust-exposed group were injected intraperitoneally (i.p.) with 1 g/kg DIPN, l/wk for 3 weeks. Also one clean-air group and one exhaust-group were not injected. The animals were necropsied at 6, 12, 18, and 24 months. No lung tumors were found in any of the rats that did not receive injections. Of those animals receiving injections, 12 animals breathing clean air had tumors and 15 animals breathing diesel exhaust had tumors in the final six months of the study. In that same period, the incidence of lung tumors was higher in the diesel exhaust + DIPN exposure group (7/18) compared to the clean air + DIPN group (4/21), but this difference did not reach statistical significance in this very small study.

6.1.3 INTRATRACHEAL ADMINISTRATION

The studies evaluating the carcinogenic activity of diesel exhaust or its components, administered by intratracheal instillation or lung implantation, are summarized in Table 6.3.a-b.

6.1.3.1 STUDIES IN HAMSTERS

Investigators in two of the studies in hamsters examined the effects of intratracheally (i.t.) administered diesel exhaust particles or organic extracts of diesel exhaust particles (Shefner *et al.*, 1982, 1985; Kunitake *et al*., 1986). As in inhalation studies in this species, no significant increase in tumors was observed.

Sato *et al*. (1986) investigated the carcinogenic potential of 1,6-dinitropyrene (1,6-DNP), a potent mutagenic component of diesel exhaust, in hamsters. Ten week old animals were dosed with saline or 0.5 mg 1,6-DNP by i.t. administration once weekly for 26 weeks. After an additional 22 weeks, the lung adenocarcinomas and myeloid leukemia incidences, 19/20 (95%) and 12/20 (60%), respectively, in the l,6-DNP treated group were much higher than the incidence observed in the control group, 0/20 for both of these cancers.

6.1.3.2 STUDIES IN RATS

Kawabata *et al*. (1986) examined the effects of diesel exhaust particulates (1 mg/rat), activated carbon (1 mg/rat), and vehicle solution, administered intratracheally, or no treatment on the incidence of lung tumors in female Fischer 344 rats. The various treatments were administered once per week for 10 weeks. Following the treatment period the animals were observed for 30 months.

The lung tumor incidence was: diesel exhaust particulate - 31/42 (74%); activated carbon - 11/23 (48%); vehicle control - 1/23 (4%); and no treatment - 0/44. Diesel exhaust and activated carbon were significantly elevated compared to controls. Diesel exhaust lung tumor incidences, particularly malignant tumors, were also significantly higher than those after activated carbon treatment.

By implantation in the lung, Grimmer *et al*. (1987) examined the carcinogenicity of fractions of diesel exhaust obtained by a special condensation and filtration method. The condenser and the filter were extracted with acetone. The resulting extracts and the condensate were evaporated and freeze dried. Twelve different treatment groups, each with 35 Osborne-Mendel rats, were then tested. Three groups received BaP as a reference. Except for the untreated controls, the substance to be tested was dissolved in acetone, and to that solution was added beeswax and trioctanoin. The acetone was then evaporated at reduced pressure, leaving the agent in a vehicle that was injected into the lung, so as to form, upon cooling, a pellet, from which the test substance could diffuse.

In the initial fraction of the exhaust residue the hydrophobic yield was 75% of the whole. This fraction, using a treatment of 20 mg/mouse, produced 5 carcinomas in the group of 35, compared to no tumors in the untreated group or the vehicle control. The hydrophobic fraction was further separated into several subfractions: 1) non-aromatic and 2-3 ring polyaromatic compounds (PACs); 2) 4-7 ring polyaromatic hydrocarbons (PAHs); 3) polar PACs; and 4) nitro-PAHs. Subfraction 2, PAHs consisting of 4-7 rings, was found to be the most potent subfraction; when proportionately dosed, it produced 6 carcinomas in a group of 35 rats. The only other subfraction producing malignant tumors was subfraction 4, the nitro-PAHs, which produced 1 carcinoma in the 35 rats. The remaining subfractions, subfraction 1 (non-aromatic and 2-3 ring PACs) and subfraction 3 (polar PACs), produced only 1 benign (adenoma) tumor. It is interesting to note that reconstituting the hydrophobic subfractions produced the same carcinogenic response (7 carcinomas in 35 rats) as the main (i.e. unfractionated) hydrophobic fraction. These data suggest that nearly all of the carcinogenic activity in this assay of diesel exhaust extract originated from the 4-7 ring PAH subfraction.

Pott *et al*. (1994) examined the ability of several fibrous and nonfibrous dusts, including diesel exhaust particulate matter, carbon black, TiO_2 (anatase or rutile) and activated charcoal, to induce lung tumors by intratracheal instillation in female Wistar rats. The animals were 12-15 weeks of age at the start of exposure. Particle physical data and the method used to generate the diesel exhaust particulate matter used in the study were not described. Two different types of diesel exhaust particulate matter were evaluated (diesel soot A and B), but the differences between the types were not discussed. Each instillation described here was of 3 mg. The diesel soot A group (40 animals) received 15 instillations; the diesel soot B groups received either 10 instillations (58 animals) or 20 instillations (38 animals). The carbon black (37 animals) group received 15 instillations; the activated charcoal groups received 10 (37 animals) and 20 (39 animals) instillations, respectively. The anatase and rutile TiO_2 exposure groups (39 animals each) received 15 and 20 instillations, respectively. All instillations were given at weekly intervals. Vehicle control groups were included (15 and 20 instillations, respectively). Surviving animals were sacrificed at no later than 131 weeks. Body weight and mortality data were not provided. Total (benign and malignant) lung tumor incidences were similar for the diesel soot A (65%), diesel soot B low dose (60%) and high dose (66%), and carbon black (65%) exposure groups. Total lung tumor incidences for the activated charcoal low dose (27%) and high dose (36%) exposure groups and the TiO_2 anatase (5%) and rutile (3%) groups were comparatively lower. No tumors were noted in the vehicle control groups. Squamous cysts were included in the total tumor count.

Female Wistar rats (7 weeks old at start of treatment) were exposed to diesel soot ($DS_{original}$), toluene-extracted diesel soot (DS), toluene-extracted Printex 90 (carbon black) (Pr), toluene-extracted Lamp Black 101 (carbon black) (LB), benzo[a]pyrene (BaP), DS + BaP, or Pr + BaP by intratracheal instillation (Dasenbrock *et al*., 1996). The total dose/animal was 15 mg divided over 15-16 weekly instillations, with the exception of 30 mg toluene-extracted diesel soot (DS_{30}) and benzo[a]pyrene (BaP_{30}) groups. A vehicle control group was included. All surviving animals were sacrificed 800 days after the start of treatment. Sample size for tumor evaluation was 48 animals for all groups except for the controls and the BaP_{30} group (47 animals). No lung tumors were observed in the control animals. Benign and malignant lung tumor incidences for treatment groups were as follows (percent incidence in parentheses): $DS_{original}$ 8/48 (17%); DS_{30} 10/48 (21%); DS 2/48 (4%); Pr 10/48 (21%); LB 4/48 (8%); BaP_{30} 43/47 (90%); BaP 12/48 (25%); DS + BaP 4/48 (8%); Pr + BaP 13/48 (27%). The $DS_{original}$, DS_{30}, Pr, BaP_{30}, BaP and Pr + BaP treatment groups demonstrated tumor incidences significantly greater than that of controls. The lung tumor incidence for the unextracted diesel group ($DS_{original}$) was also significantly greater than that of the extracted diesel soot (DS) group. However, the authors included cystic keratinizing epitheliomas (squamous cysts) in their count of benign tumors. If these lesions were removed from the tumor count, there would be no significant difference in tumor incidence between the $DS_{original}$ and DS groups.

6.1.3.3 STUDIES IN MICE

Male ICR mice were exposed by intratracheal instillation (one treatment/week for 10 weeks) to 0.1 mg of either titanium dioxide (TiO_2), DEP, or hexane/benzene/methanol-washed DEP (WDEP) (Ichinose *et al*., 1997a). Treatment groups (including controls) consisted of 33 animals. All surviving animals were sacrificed at 12 months. Necropsies were performed on all animals. Six animals from each group were used for lung 8-OHdG adduct analysis; lung histological examinations were performed on all other animals. The numbers of animals examined for tumors were 26 animals for DEP, and 27 animals for all other groups. Total lung tumor incidence after 12 months (adenomas and adenocarcinomas) was significantly increased in the DEP treatment group (35%) but not in the WDEP (26%) or TiO_2 (16%) treatment groups when compared to controls (11%). As described in Chapter 5, animal lung DNA was also assayed for the presence of the oxidative DNA adduct 8-hydroxydeoxyguanosine (8-OHdG). 8-OHdG levels were increased in the WDEP and DEP groups but not in the TiO_2 group when compared to controls. Regression analysis indicated that total lung tumor incidence was significantly correlated with 8-OHdG levels ($r = 0.99$, $p < 0.01$).

6.1.4 SKIN PAINTING OF DIESEL EXHAUST OR DIESEL EXHAUST COMPONENTS.

Summaries of the studies evaluating the carcinogenic potential of diesel exhaust or diesel exhaust components following dermal application are presented below. Administration by skin painting has limited relevance to diesel exhaust emissions and human exposure, therefore the following is simply a brief discussion of the results; these studies are also listed in Table 6.4.

Nesnow *et al*. (1982a; 1982b; 1983) in a series of reports has compared the tumor initiation potential of a variety of diesel engine exhaust particulate extracts in mouse skin-painting studies, with TPA as the promoter. The diesel engine samples varied widely in the tumorigenic responses they produced. Because the fuel, driving cycle and collection and extraction procedures used in the production of the light duty diesel samples were similar, the difference in tumor responses are most likely because of differences in engine design and operating characteristics (Nesnow *et al*., 1982b). Mouse skin has been reported to be quite sensitive to the tumorigenic effects of PAHs, however, the tumor response data presented in these studies cannot be explained solely by the BaP content. There was no significant relationship between the mean number of papillomas per mouse and BaP content in each sample. According to the authors, BaP content could account for only 20-30 percent of the activity seen and other components of the samples seem to be playing a role in the tumorigenic activity.

Nesnow *et al*. (1982a; 1982b) also conducted mouse skin-painting studies under a complete carcinogenesis protocol. This protocol is a test for agents exhibiting both tumor initiating and tumor promoting activities. The diesel engine samples were essentially inactive as complete carcinogens at the doses applied.

Depass *et al*. (1982) conducted tumor initiation, tumor promotion, and complete carcinogen evaluations of diesel exhaust particulate suspensions as well as diesel particulate extracts. PMA (phorbol 12-myristate 13-acetate) was used as the promoting agent in the initiation studies. BaP was used as the initiating agent in the promotion studies. Diesel particulate was generated by an Oldsmobile 350D diesel engine. The results of these studies suggested that Oldsmobile diesel exhaust particulate and diesel exhaust particulate extract have little tumor initiating, promoting or complete carcinogenic activity under the conditions of this bioassay.

Kunitake *et al*. (1986) studied the carcinogenic activity of heavy-duty diesel engine exhaust extracts (HDE). A mouse-skin protocol evaluated initiation potential. The skin tumors produced were predominantly squamous cell papillomas. Skin carcinomas were not found in any treatment group. The percent of tumor bearing mice increased with dose in the HDE (0-8%) and in the HDE + BaP (2-10%) treatment groups. Based on the carcinogenic potential of BaP, the authors had anticipated a much higher tumor incidence in the HDE + BaP treatment group.

Nesnow *et al*. (1984) studied the initiation potential of l-NP and a mixture of its nitrated products, 1,3-dinitropyrene (DNP), 1,6-DNP, and 1,8-DNP (1:1.94:1.95), utilizing the SENCAR mouse skin bioassay. Compared to controls l-NP did not induce papilloma formation over a dose range of 0-3.0 mg/mouse after 30 weeks of promotion with TPA. The mixture of dinitropyrenes applied over a dose range of 0 - 2.0 mg/mouse resulted in a significant induction of papillomas at 30 weeks at the highest dose.

El-Bayoumy *et al*. (1982) examined the tumor-initiating activity of specific diesel exhaust components using a mouse skin painting bioassay. The eight components evaluated were: BaP, chrysene, perylene, pyrene and their nitro-derivatives 6-nitrobenzo(a)pyrene (6-NBaP), 6-nitrochrysene (6-NC), 3-nitroperylene (3-NPerl), and 1-nitropyrene (l-NP). BaP produced tumors in 90% of the mice and was significantly different from controls and 6-NBaP. 6-NBaP

did not produce a significant increase in the number of tumors compared to controls. Neither pyrene or l-NP exhibited tumorigenic activity at the concentrations tested compared to controls. 6-NC induced tumors in 60% of the mice, but was significantly less tumorigenic than chrysene (100%). Both chrysene and 6-NC were significantly different than the controls. 3-NPerl induced tumors in 42% of the mice and was significantly more active than perylene and controls. Among the nitro-compounds tested, only 3-NPerl was more tumorigenic than its parent hydrocarbon.

6.1.5 SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

Since the mid-1980's, chronic inhalation studies have consistently demonstrated significant increases in lung tumors in rats exposed to unfiltered diesel exhaust at particulate concentrations of greater or equal to 2.2 mg/m^3 and with exposure times of approximately two years. Nonsignificant increases in lung tumor incidence have also been shown at particulate concentrations of between 0.35 mg/m^3 and 2.2 mg/m^3 (Ishinishi *et al*., 1986a; Mauderly *et al*., 1987a). All except two studies examining the carcinogenicity of filtered diesel exhaust have reported negative results. The study by Heinrich *et al*. (1986a) conducted in female NMRI mice demonstrated an increase in lung tumor incidence. The study by Iwai *et al*. (1986) reported that filtered and unfiltered diesel exhaust increased the incidence of splenic malignant lymphomas in female Fischer 344 rats. Additionally, equivocal results were reported in female NMRI mice by Heinrich *et al*. (1995). To date, the results of the two positive studies on filtered diesel exhaust have not been confirmed by other investigators.

Evidence for the carcinogenic importance of the particulate fraction of diesel exhaust comes from two studies in rats which found that diesel exhaust that had the particulate fraction removed by filtration did not demonstrate carcinogenicity (Heinrich *et al*., 1986a; Brightwell *et al*., 1989) whereas the unfiltered diesel exhaust did demonstrate carcinogenicity. Furthermore, preliminary evidence shows that carbon-black (elemental carbon) particulate, without an appreciable organic coating, can induce lung tumors in rats at approximately the same carcinogenicity per unit of mass as diesel exhaust (Heinrich *et al*., 1995; Nikula *et al*., 1995). Thus, OEHHA is focusing on the particulate matter in the diesel exhaust as the primary agent of carcinogenicity; the specific engine source or fuel would appear to have little impact on the tumorigenic response in the animal model.

The rat bioassay data are insufficient to determine if sex differences exist in the development of lung tumors in rats after exposure to diesel exhaust. Four of the seven positive diesel exhaust inhalation rat bioassays used both male and female F344 rats. Brightwell *et al*. (1986; 1989) reported total lung tumor incidences of 44% and 96% in males and females, respectively, in animals sacrificed after the end of the 24 month exposure period. However, mortality data was not provided, so it cannot be determined if differences in survival between the two sexes affected tumor incidence rates. Ishinishi *et al*. (1988) found total lung tumor incidence to be greater in males than females at the highest two exposure levels for each of the two diesel engine types tested. Mortality rates were similar for both sexes. Mauderly *et al*. (1986) reported similar mortality rates and total lung tumor prevalence rates for male and female rats. Nikula *et al*. (1995) noted that female rats were more susceptible than male rats to developing lung tumors after exposure to diesel exhaust; total lung tumor incidence rates for male rats were 4.8% and

8.5% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively. Corresponding total lung tumor incidence rates for female rats were 7.6% and 27.4% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively. However, mortality rates for male rats were also greater than female rats. At 23 months, percent cumulative mortality in males was 86% and 94% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively; percent cumulative mortality in females was 69% and 73% for the 2.5 and 6.5 mg/m^3 diesel exhaust groups, respectively. As described in Section 6.1.1.2 of this document, the authors noted that logistic regression modeling did not show significant differences between the tumor responses to diesel exhaust and carbon black for either sex. However, they also noted that slope estimate errors in the logistic regression models were large; the errors were particularly large for the males, because of their much shorter lifespan compared to the females. Lung tumor incidence in the females increased rapidly towards the end of their lifespan, when most of the males had died of other causes. Also, the lack of lung tumors in the control females may have increased the estimated slopes for the treatment group females. The authors stated that these factors make it difficult to determine if there were true gender differences in neoplastic incidence or differences in the responses of males to diesel exhaust and carbon black.

Hamsters do not show carcinogenic effects from inhalation of diesel exhaust under the study protocols evaluated thus far. Hamsters treated with diethylnitrosamine or benzo[a]pyrene (but not exposed to diesel exhaust) unexpectedly showed only low incidences of respiratory tract tumors (Heinrich *et al*., 1986a). Therefore, low pulmonary tract sensitivity to genotoxic carcinogens could potentially account for the lack of carcinogenic response to diesel exhaust in hamsters. The rat appears to be the most responsive laboratory species to inhaled diesel exhaust particulate.

Diesel exhaust exposure has not been demonstrated to cause increased tumor incidence in monkeys in the single study performed to date (Lewis *et al*., 1986, 1989). However, the low number of animals used and the less than lifetime exposure employed render the results of this study inadequate for determining cancer risks due to diesel exhaust exposure.

Investigations of the effect of diesel exhaust exposure with co-administration of specific known carcinogens have not shown significant synergism. Substances investigated for a combined effect in mice are the carcinogens, BHT, BaP or DBahA. Substances investigated in rats are DPN and DIPN.

The carcinogenicity of diesel exhaust following intratracheal administration has been investigated. Studies utilizing diesel exhaust particulate have produced negative results in hamsters but positive results in rats and mice. This pattern of response is similar to that observed following inhalation exposure. Diesel exhaust condensates have also produced a positive tumorigenic response in the rat following intratracheal administration.

Skin painting of diesel exhaust extracts has produced a wide variety of tumorigenic responses. The authors suggested that the differences in tumor responses were most likely due to differences in engine design and operating characteristics. Mouse skin has been reported to be quite sensitive to tumorigenic effects of PAHs; however, only 20-30 percent of the activity of diesel

exhaust extracts could be attributed to BaP content. A variety of specific diesel exhaust components have also exhibited tumorigenic potential following skin painting.

6.1.6 MECHANISM OF ACTION OF DIESEL EXHAUST-INDUCED RAT LUNG TUMOR INDUCTION

The mechanism(s) of action by which diesel exhaust induces lung tumors in rats has not been established. Several hypotheses have been proposed. One hypothesis is that PAHs and nitroPAHs contained either in the semivolatile phase or adsorbed on the surface of diesel exhaust particulate matter induce lung tumors via a genotoxic mechanism. Evidence in support of this hypothesis include 1) the demonstrated genotoxicity of whole diesel exhaust, the semivolatile phase of diesel exhaust and extracts (in either organic solvents or simulated physiological fluids) of diesel exhaust particulate matter (see Chapter 5); 2) the bioavailability of PAHs from diesel exhaust (see Chapter 3). Additionally, several investigators have shown that lung tumor incidences were increased when the PAH benzo[a]pyrene (BaP) adsorbed on particles (Fe_20_3 or carbon) was intratracheally instilled compared to instillation of the pure compound (Sellakumar *et al*., 1976; Henry *et al*., 1975). However, this hypothesis is not supported by the finding that the tumorigenic potencies of the nongenotoxic particulate substances carbon black and TiO_2 in rats exposed by inhalation are similar to that of diesel exhaust.

The existence of a previously unknown strongly carcinogenic substance(s) is another possible hypothesis. Given the fact that there are at least several hundred organic compounds present in diesel exhaust, it is possible that there may be an unknown compound(s) among them that has a strong carcinogenic potency.

A third hypothesis is that diesel exhaust induces oxidative DNA damage by a mechanism other than particle-induced inflammation. Formation of 8-hydroxydeoxyguanosine (8-OHdG) adducts leads to G:C to T:A transversions unless repaired prior to replication, and may be promutagenic (Nagashima *et al*., 1995). Pulmonary toxicity in mice exposed to diesel exhaust particulate matter has been shown to be mitigated by pretreatment with the lung antioxidant enzyme superoxide dismutase (SOD)(Sagai *et al*., 1993). This suggests that active oxygen species are produced as a result of exposure to diesel exhaust. In the same study, lung antioxidant enzyme (SOD, glutathione-S-transferase, glutathione peroxidase) activities were significantly reduced in mice exposed to diesel exhaust particles by intratracheal instillation. The antioxidant enzyme catalase has also been demonstrated *in vitro* to be inhibited by diesel exhaust particulate matter. Additionally, exposure of mice to diesel exhaust particles by intratracheal instillation resulted in a significant increase (approximately 3-fold) in mouse lung DNA 8-OHdG adducts (Nagashima *et al*., 1995). Ichinose *et al*. (1997) found that exposure of mice to either hexane/benzene/methanol-washed diesel exhaust particulate matter (WDEP), diesel exhaust particulate matter (DEP), or titanium dioxide (TiO_2) by intratracheal instillation resulted in a significant induction of both 8-OH-dG adducts, by DEP and WDEP, with DEP causing a higher level of adducts than WDEP, and lung tumors in the DEP group, but not the WDEP group. TiO_2 did not cause a significant increase in either 8-OH-dG adducts or lung tumors. Regression analysis indicated that total lung tumor incidence was significantly correlated with 8-OHdG levels. These data suggest that induction of oxidative DNA damage by diesel exhaust may lead

to mutagenicity, which could then play a part in the initiation of tumorigenesis. It should be noted that no data exists on the ability of carbon black to induce oxidative DNA damage.

A fourth hypothesis is that the inflammatory response to the accumulating exhaust particles may promote cell proliferation. This would increase the probability that any existing DNA damage would result in a heritable mutation before repair could take place, and would therefore increase the risk of tumor induction. DNA damage could result from mutagens in the organic compounds that coat the particle as it arrives at the lung surface, but that eventually leave the particle. Increased particle accumulation in rat lung results in impaired particle clearance and therefore increased retention time, leading to potentially increased mutagen exposure. Alternatively, DNA damage could be caused by other carcinogens that happen to be present, by an oxidative mechanism not requiring inflammation, or by the inflammatory response to the accumulated exhaust particles. Inhalation of the nongenotoxic particulate substances carbon black and TiO_2 have also been demonstrated to be approximately as potent as diesel exhaust per unit of mass in inducing lung tumors in rats. In addition to chronic inflammation, exposure to diesel exhaust and carbon black also results in epithelial hyperplasia (Nikula *et al*., 1995). These phenomena can occur early in the exposure period (3-6 months of exposure) at exposure levels that cause tumors.

It has been proposed that rat lung tumors caused by chronic particle exposure occur due to increased lung particle burden which exceeds clearance capacity resulting in particle-elicited inflammation and increased epithelial cell proliferation in the pulmonary region of the rat lung (Oberdörster, 1994; Nikula *et al*., 1995; Driscoll, 1996). Particle phagocytosis increases active oxygen species generation and inflammatory cytokine release by macrophages. Polymorphonuclear leukocytes (PMNs) also produce active oxygen species in response to membrane-reactive particles. Lung particle deposition can result in both an increase in the alveolar macrophage (AM) population and efflux of PMNs from the vascular compartment into the alveolar space compartment. PMN recovery by lavage from the lungs of diesel exhaust-exposed rats has been observed to increase with both increasing exposure duration and particle concentration (Strom, 1984). Activated phagocytic cells have been observed to induce genotoxicity in a variety of *in vitro* short-term assays; in most cases, active oxygen species have been implicated as being involved in the observed genotoxicity (Driscoll, 1996). Continued high particle exposure levels with resulting excessive lung particle burden could result in the anti-inflammatory protective mechanisms (antioxidants, oxidant metabolizing enzymes, cytokine inhibitors, etc.) of the lung being overwhelmed with resulting tissue damage, cell proliferation and possibly genotoxicity. However, it has been noted (Mauderly, 1994a) that carcinogenicity studies with another insoluble particle (copier toner) have been performed (Muhle *et al*., 1991) that resulted in particle clearance overload (Bellmann *et al*., 1992) without induction of neoplasia.

Exposure levels of carbon black which cause chronic inflammation in rats also have been demonstrated to induce mutations at the hprt locus of rat lung type II alveolar epithelial cells (Driscoll *et al*., 1996). Male Fischer 344 rats were exposed 6 hr/d, 5 d/wk for up to 13 weeks to 0, 1.1, 7.1 or 52.8 mg/m^3 carbon black. After 13 weeks of exposure, carbon black lung burdens in the low, medium and high dose groups were 354, 1826 and 7861 µg/lung, respectively. Lung

clearance of carbon black was impaired in the medium and high dose groups. Total cell number (macrophages, neutrophils and lymphocytes) found in bronchoalveolar lavage fluid was increased after 6.5 and 13 weeks of exposure in the high dose group but not the medium or low dose groups; however, the proportion of neutrophils in the total cell population increased in the medium and high dose exposure groups. Expression of mRNA coding for the chemotactic cytokines macrophage inflammatory protein 2 (MIP-2) and monocyte chemotactic protein 1 (MCP-1) was increased after both 6.5 and 13 weeks exposure in the medium and high dose exposure groups. Type II alveolar epithelial cells were isolated from exposed rats and controls and grown in primary culture to determine mutation frequencies at the hprt locus using 6-thioguanine (6-TG) resistance as a biomarker. Hprt mutation frequencies were significantly increased in cells obtained from the medium and high dose groups after 13 weeks of exposure. These data indicate that exposure of rats to carbon black at concentrations which have been shown to induce lung tumors also cause an increase in both the production of chemotactic cytokines by the lungs and in mutation induction in a cell type believed to be at least one of the progenitors of lung tumors observed after carbon black exposure. This suggests that the increased rat lung tumors observed after carbon black exposure may be initiated by genotoxicity resulting from chronic inflammation mediated by increased chemotactic cytokine production. This also raises the possibility, but does not provide direct proof, that diesel exhaust-induced rat lung tumors may be initiated via a similar mechanism.

Information on potential differences and similarities in the mechanisms of tumor induction by diesel exhaust and carbon black could potentially be provided by an analysis of the mutational spectra in those tumors. Diesel exhaust- and carbon black-induced rat lung tumors (adenocarcinomas, squamous cell carcinomas, adenosquamous carcinomas) generated in the study done by Nikula *et al*. (1995) were analyzed for mutations in the p53 and K-ras genes (Swafford *et al*., 1995). Differences in mutation frequencies between diesel exhaust-induced and carbon black-induced tumors would have indicated potential differences in the mechanism of initiation. However, no increases were found in p53 mutations in tumors from either treatment group, and only a low frequency of K-ras mutations was observed (3/50 - 1 in the carbon black group, 2 in the diesel exhaust group). Rosenkranz (1996) noted that the induction of p53 mutations in rat lung tumors by genotoxic carcinogens is much lower than in humans and mice; additionally, rat K-ras is not mutated by all genotoxins, and some tumors induced by putative non-genotoxic carcinogens contain mutated K-ras. These data indicate that the currently available mutational spectra data for diesel exhaust and carbon black are insufficient for use in drawing inferences about the mechanism of lung tumor induction for those two substances.

The information presented above suggests that chronic inflammation resulting in macrophage and/or neutrophil-induced oxidative DNA damage resulting in mutations may be mechanistically important in the induction of lung tumors, and that cell proliferation may be mechanistically important to the promotion of lung tumors in rats exposed to high levels of diesel exhaust by inhalation. Diesel exhaust may also induce oxidative DNA damage via mechanisms other than inflammation. However, the possibility that genotoxicity due to the PAH and nitroPAH content of diesel exhaust may play a role in the induction of lung tumors in rats at lower levels of diesel exhaust cannot be excluded; the diesel exhaust carcinogenicity bioassays performed to date have not had sufficient power to detect the carcinogenicity of diesel exhaust at low doses not

exceeding a putative threshold. Existing studies cannot detect the comparatively low levels of tumor formation of interest. Mauderly (1994c) noted that small increases in lung tumor incidence have been observed in rats at diesel exhaust concentrations which do not induce cell proliferation or fibrosis, but group sizes have been insufficient (maximum of approximately 200) to permit the significance of the small tumor incidence increases to be evaluated with confidence.

Additionally, a recent report by Borm *et al*. (1997) indicates that incubating rat lung epithelial-derived cells with human polymorphonuclear lymphocytes (PMN) (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to benzo[a]pyrene; addition of more activated PMN in relation to the number of lung cells further increased adduct formation in a dose-dependent manner. The authors suggest that "an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particulates." These data raise the possibility that low dose diesel exhaust exposure may result in levels of neutrophil influx which would not necessarily be detectable via histopathological examination as acute inflammation but which might be effective at amplifying any potential diesel exhaust genotoxic effect.

It should also be noted that some parameters of the "particle overload" hypothesis are incompletely characterized. Alveolar type II cell epithelial hyperplasia has been noted after diesel exhaust exposure, but the measures of cell proliferation used were relatively crude and unsuitable for use in a quantitative estimate of cell proliferation as would be required for biologically-based modeling. It should also be noted that uncertainties exist regarding the magnitude and biological importance of particle overload for diesel exhaust-induced rat lung carcinogenicity. Mauderly *et al*. (1994) included data from a rat bioassay on the number of neutrophils/mL present in bronchoalveolar lavage fluid from the exposed and control animals (males and females combined). Active oxygen species generated by activated neutrophils are one component of the inflammatory response to diesel exhaust exposure that might be mechanistically important to the induction of tumorigenesis. The number of neutrophils was increased approximately 50-75% for the high carbon black group compared to the low carbon black group; the increase for the high diesel exhaust group was 20-40% compared to the low diesel exhaust group. However, the tumor incidence (males and females combined) for the high carbon black and diesel exhaust groups were approximately 3-fold greater than that for the low carbon black and diesel exhaust groups, respectively. Similarly, the differences in the severity scores for alveolar macrophage hyperplasia and alveolar epithelial hyperplasia in rats that died or were killed after 18 months of exposure between the low and high diesel exhaust groups (approximately 25 and 20%, respectively) do not correlate well with tumor incidence. It would be expected that a better correlation between tumor incidence and indices of inflammation and cell proliferation would exist if diesel exhaust-induced rat lung tumors were solely due to particle overload.

Hattis and Silver (1994) examined lung burden data from diesel exhaust rat carcinogenicity studies and came to the conclusion that "there is continuing accumulation of diesel-derived dust in the lungs of rats throughout life, even at low doses". They also found that this was not predicted by models developed to represent diesel exhaust particulate matter accumulation under "overload" versus non-overload conditions. Finally, they have found that at high diesel exhaust

exposure levels, the increase in the ratio of internal diesel exhaust particulate matter burden to external exposure is not very large, being slightly larger than a factor of 2 at most, and state that "Although dust overloading is a real phenomenon, it is not a very large effect and thus would not be expected to give rise to dramatically lowered estimates of risk at low exposure levels."

Diesel exhaust-induced chronic inflammation may have a threshold of effect. However, genotoxicity induced by the PAH/nitroPAH content of diesel exhaust would not be expected to have an effect threshold. This suggests that both potentially threshold and nonthreshold mechanisms may play a role in the induction of rat lung tumors after exposure to diesel exhaust.

6.2 EPIDEMIOLOGICAL STUDIES

The epidemiological evidence concerning the carcinogenicity of diesel exhaust primarily involves cancers of the lung and bladder. The review focuses first on studies of lung cancer (Sections 6.2.1 and 6.2.2) and then turns to those of bladder cancer (Section 6.2.3). The evidence for causation of lung cancer is then assessed using criteria for causal inference from epidemiological studies (Section 6.2.4). Because there are no epidemiological studies involving industrial hygiene measurements concurrent with the exposures of the study populations, exposure has typically been defined by the surrogate measures of usual occupation or job classification within an industry. Summary findings from each study are presented in Table 6.5.

This chapter provides evidence consistent with a causal relationship between occupational diesel exhaust exposure and lung cancer. A lengthy discussion of causal inference, including the strengths and limitations of the underlying data, can be found in Section 6.2.4. The evidence linking diesel exposure and bladder cancer is not as extensive or compelling. In summary, the key findings relating lung cancer and occupational exposure to diesel exhaust are as follows: the majority of studies examining the diesel exhaust-lung cancer association have reported elevated estimates of relative risk, many of which are statistically significant. The consistency of these findings is unlikely to be due to chance. Moreover, with the possible exception of some studies that did not take smoking into account, the results are unlikely to be explained by confounding or bias. This is reinforced by the results of a meta-analysis undertaken by OEHHA staff (Appendix C, summarized in section 6.2.2), in which statistically significant pooled estimates of relative risk persisted through numerous subset and sensitivity analyses. The most important potential confounder is cigarette smoking, which was measured and controlled for in multiple studies: in the meta-analysis the pooled relative risk estimate for studies that adjusted for smoking was 1.43 (95% C.I. = 1.31-1.57). In addition, several studies provide evidence of exposure-response relationships. The strength of the associations reported is typically within the range considered "weak" in epidemiology (i.e., estimates of relative risk between 1 and 2); nonetheless, this is not a bar to causal inference as long as other criteria are met, as discussed in Section 6.2.4. Thus, a reasonable and very likely explanation for the increased risks of lung cancer observed in the occupational epidemiological studies is a causal association between diesel exhaust exposure and lung cancer.

6.2.1 REVIEW OF LUNG CANCER STUDIES

The question of whether diesel exhaust causes lung cancer has been addressed by both industry-based cohort and case-control studies as well as population-based studies of lung cancer. In the following subsections, the review of the lung cancer studies has been divided into five parts focusing on studies of : (1) truck drivers, (2) transport and equipment workers, (3) dock workers, (4) railway workers, and (5) other miscellaneous occupations involving diesel exhaust exposure.

6.2.1.1 STUDIES OF LUNG CANCER AMONG TRUCK DRIVERS

Truck drivers, as a group, have been thought to experience elevated exposures to diesel exhaust. Although studies of cancer among truck drivers have not included actual measurements of diesel

exhaust exposure, auxiliary studies provide some relevant data. Ziskind *et al*. (1978) reported elevated concentrations of engine combustion products, including NO_2, NO and CO_2, in truck cabs. Zaebst *et al*. (1991) reported U.S. long distance drivers to have five-fold greater total carbon exposure compared to residential exposures (at least one mile from any major highway). In a study of Swiss drivers, Guillemin *et al*. (1992) found elevated levels of respirable particles in the diesel trucks of local drivers (0.26 mg/m^3) but not in the truck cabs of long-distance drivers (0.10 mg/m^3) or in the offices of the administrative worker controls (0.11 mg/m^3). However, it was not clear what proportion of the elevated value was due to vehicular self-pollution (entrainment of the study trucks' engine exhaust) versus ambient urban pollution.

Furthermore, information on smoking habits is often unavailable, particularly in cohort mortality studies, although there is evidence that truck drivers tend to have a high smoking prevalence (Wynder and Higgins, 1986; Williams *et al*., 1977). In the small number of drivers surveyed by Guillemin *et al*. (1992) (n=15), smoking did not significantly influence either the measured total dust or the total polyaromatic hydrocarbons for the drivers; however, large standard deviations suggest a wide range of values. Steenland *et al*. (1992) reported the findings of an industrial hygiene survey of the American trucking industry. Long-haul road drivers had an exposure of 5.1 $\mu g/m^3$ of elemental carbon, compared to a roadway background of 3.4 $\mu g/m^3$ and a residential background of 1.6 $\mu g/m^3$. In studies by both Steenland *et al*. (1992) and Guillemin *et al*. (1992), the authors commented that drivers apparently received much of their current exposures from the roadway background, rather than directly from engine exhaust.

Menck and Henderson (1976) carried out a study of occupational differences in lung cancer rates in Los Angeles County. Subjects for the study were white males, aged 20-64, who died in the period 1968-70, and whose death certificates mentioned lung cancer. Also included were all cases of lung cancer in white males in the same age group reported to the county Cancer Surveillance Program for 1972-73. Subjects were coded according to occupation. Using estimates of occupational group population sizes for the county, expected numbers of deaths and incident cases were calculated for each specific occupation, assuming the age-specific cancer rates in each occupation would be the same as those for all occupations. Occupation-specific mortality ratios were calculated after combining the deaths and incident cases. The specific mortality ratio for lung cancer among truck drivers was 1.65 ($p<0.01$). No information on smoking was available for this study.

Decoufle *et al*. (1977) conducted a case-control study of cancer patients admitted to Roswell Park Memorial Institute between 1956 and 1965. A variety of risk estimates for a range of cancers were calculated using other noncancer patients as controls. No significant increase in the risk for lung cancer was found for individuals ever employed as truck [and tractor] drivers (odds ratio (OR) = 1.07, $p>0.05$, 56 cases) or employed at least five years (OR = 0.89, $p>0.05$, 50 cases [combined with bus and taxi drivers]).

In their analysis of data from the Third National Cancer Survey (TNCS), Williams *et al*. (1977) reported a weak association between truck driving and lung cancer. The TNCS involved interviews of 7,518 patients from eight, mainly urban, areas of the U.S. The interviews included

questions on lifetime employment histories, smoking habits and socioeconomic status (SES). The principal analysis involved testing for associations between main lifetime employment in each of 202 employment categories with cancer occurring at 29 specific sites. For each cancer site, patients with cancers at all 28 other sites were combined as a control group. Appropriate adjustments were made for confounding factors, including smoking and socio-economic status (SES). After controlling for race, education, geographic location, alcohol consumption, and tobacco use among male truck drivers, the OR for lung cancer was 1.52, based on 22 cases. Although the report did not present confidence limits, this OR was not statistically significant at $\alpha = 0.05$. An important weakness of this study was the low questionnaire response rate (57%).

Luepker and Smith (1978) studied the mortality experience over a three-month period in the International Brotherhood of Teamsters, whose members are mainly truck drivers. Overall mortality showed evidence of a healthy worker effect, with an SMR of 0.74. In contrast, mortality from respiratory cancers was slightly elevated, with a standardized mortality ratio (SMR) of 1.21 ($p<0.01$) for the entire cohort, and a SMR of 1.37 ($p<0.001$) for the 50-59 year age group (n = 48,358). No separate analysis was presented for truck drivers alone, and information on smoking habits was not available. However, there was no elevation of mortality from cardiovascular disease, chronic bronchitis or emphysema, which would otherwise be expected to accompany a high smoking prevalence. Additional limitations of this study include the exclusion of retirees and members with lapsed benefits, as well as a short follow-up period.

Ahlberg *et al*. (1981) identified a cohort of Swedish truck drivers from the 1960 national census (n = 34,027). A comparison population was formed with other blue-collar workers without known exposure to petroleum or chemical products (n = 686,708). Between 1961 and 73, 161 lung cancers were registered within the cohort. For all truck drivers, the relative risk (RR) of lung cancer was 1.33 (95% C.I. = 1.13-1.56), while Stockholm drivers had a higher risk (RR = 1.62, 95% C.I. = 1.15-2.28). Although no individual data on smoking were available, a questionnaire survey of professional drivers in Stockholm found that 31% of truck drivers smoked, excluding those who drove fuel tank trucks. For comparison, the authors cited an unpublished report indicating a smoking prevalence of 40% in Stockholm.

Milne *et al*. (1983) performed a case-control study of all lung cancer deaths occurring in Alameda County, California, in the period 1958-1962. The study group consisted of 925 lung cancer cases and 6,420 other cancer controls, all of whom were coded according to occupation as listed on the death certificate. Employment as a truck driver (23 cases, 53 controls) was positively associated with lung cancer (OR = 1.6, $p<0.05$). However, when cancers associated with occupational factors (cancers of the pancreas, nasal sinus, kidney, bladder, bone and hematopoietic system) were excluded from the control group, the odds ratio for truck drivers decreased to 1.3, which was not statistically significant at the 95% confidence level. No information on smoking habits was available.

Hall and Wynder (1984) performed a case-control study of 502 lung cancer patients, aged 20 to 80, using as controls 502 patients at the same hospitals who did not have smoking-related diseases. All cases and controls were interviewed about smoking history and "usual lifetime

occupation." Occupations were dichotomized into diesel-exposed and unexposed; warehousemen, bus drivers, truck drivers, railroad workers, and heavy equipment operators were all considered exposed. For all diesel-exposed occupations the OR was 2.0 (95% C.I. = 1.2-3.2), but decreased to 1.4 (95% C.I. = 0.8-2.4) after adjusting for smoking. The OR for truck drivers was 1.4 (95% C.I. = 0.7-2.6). However, due to the limited number of cases (n = 22), the authors did not adjust this estimate for smoking.

Boffetta *et al*. (1990) conducted a follow-up study to that reported by Hall and Wynder (1984), which included 2,584 lung cancer cases and 5,009 hospitalized controls. Exposure was assessed by both occupational titles and self-reported exposure to diesel exhaust. After controlling for smoking, asbestos exposure and education, the ORs for the occupational groups considered to have had "possible" and "probable" diesel exposure were 0.92 and 0.95, respectively. The group with the longest duration of probable exposure to diesel exhaust (>30 years) had an OR of 1.49 (95% C.I. = 0.72-3.11) based on 17 cases and 19 controls. The only occupation with sufficient subjects for separate consideration was truck driving, comprising 114 cases and 176 controls, but duration of employment data were available only for 23 cases and 27 controls. The smoking-, asbestos- and education-adjusted OR for truck drivers with greater than 30 years exposure (n = 16) was 1.17 (95% C.I. = 0.4-3.41). The small number of cases in the exposed categories limits the interpretation of the study results.

In a case-control study of male lung cancer in Northern Sweden, Damber and Larsson (1985) examined the risk to professional drivers (including truck drivers) by stratified analysis. The study included 604 cases reported to the national death registry between 1972 and 1977. Cases were each matched with another death (due to causes other than lung cancer and suicide) and a living referent. Occupational histories were obtained by interview with next-of-kin or living controls. The cases included 63 professional drivers, 35 of whom were truck drivers. The professional drivers, both cases and controls, included a greater proportion of smokers than the rest of the study population. As expected, the results of the stratified analysis showed a clear association of lung cancer with cigarette smoking habits. The analysis also suggested an association between professional driving and lung cancer. For nonsmoking cases diagnosed with lung cancer at ages less than 70, the OR was 1.9 (95% C.I. = 0.5-5.5), and for those 70 years or older at the time of diagnosis, the OR was 4.5 (95% C.I. = 1.1-16.4). Furthermore, among smokers older than age 70 the OR for truck driving was 43.3 (95% C.I. = 15.3-122.5), a significant increase above the OR for smokers who were not truck drivers, 6.7 (95% C.I. = 3.7-12.2), with both ratios using nondriving nonsmokers as the reference group. The authors suggested a synergistic interaction between smoking and driving, but the findings were inconclusive in light of the heavier smoking among truck drivers and the small numbers involved. The report indicated that diesel engines were much more common than conventional gasoline engines in the vehicles driven by this study population. However, no quantitative assessment of diesel versus gasoline exhaust exposure was provided.

In a later analysis of the same study population, Damber and Larsson (1987) calculated ORs for professional drivers as a whole, as well as for several other occupations. They reported that the unadjusted OR for professional drivers was elevated, but not significantly (OR = 1.5, 95% C.I. =

0.9-2.6). After adjusting for smoking the estimate did not attain statistical significance, even for those drivers who had worked for more than 20 years (OR = 1.2, 95% C.I. = 0.6-2.2).

Using data from the first two years of the American Cancer Society's prospective mortality study, Boffetta *et al*. (1988) analyzed the relationship between mortality and diesel exhaust exposure. The study included over 1.2 million men and women from throughout the U.S. who were enrolled in the fall of 1982. Information was obtained by questionnaire on a variety of subjects, encompassing occupational history and exposures to twelve groups of substances, including diesel exhaust and tobacco smoke. The analysis was restricted to men aged 40-79 at enrollment whose status was known at the two year follow-up, with 92,038 subjects excluded due to lack of information on diesel exposure and 14,667 excluded due to lack of information on smoking. For the group with known smoking habits and occupational exposure to diesel exhaust (n = 62,800), the relative risk for diesel exposure and lung cancer (adjusted for age and smoking) was 1.18 (95% C.I. = 0.97-1.44). In a subanalysis stratified by duration of diesel exposure for all occupations, Boffetta *et al*. reported a suggestive, although not statistically significant, exposure-response relationship by duration of employment with the relative risks of 1.05 (95% C.I. = 0.80-1.39) for 1-15 years and 1.21 (95% C.I. = 0.94-1.56) for 16 or more years of reported exposure to diesel exhaust (test for trend: $0.05 < p < 0.10$). For truck drivers overall (n = 48 cases), the smoking-adjusted relative risk for lung cancer was 1.24 (95% C.I. = 0.93-1.66), similar to the risk found in the 18 cases among truck drivers reporting diesel exhaust exposure (RR = 1.22, 95% C.I. = 0.77-1.95). There was also nonsignificant evidence of a trend of increasing lung cancer risk among truck drivers with increasing duration of exposure (for 1-15 years of exposure, RR = 0.87, 95% C.I. = 0.33-2.25 based on six exposed cases, and for 16+ years of exposure, RR = 1.33, 95% C.I. = 0.64-2.75, based on 12 exposed cases, with truck drivers reportedly not exposed to diesel exhaust as the reference category). Approximately 20% of study subjects with known smoking status were excluded from the analysis because of lack of information on diesel exhaust exposure: since this group experienced greater risks of mortality from all causes and from lung cancer than the group with known diesel exposure status, it is likely that this exclusion produced a downward bias in the estimates of relative risk.

Benhamou *et al*. (1988) conducted a study between 1976 and 1980 of 1,334 histologically confirmed cases of lung cancer among adult males and 2,409 matched controls (from an original hospital-based study of 1,625 cases and 3,091 controls). Occupations and smoking histories were determined by interview; jobs were coded to International Labor Organization classifications. The smoking-adjusted OR was 1.42 (95% C.I. = 1.07-1.89) for all professional motor vehicle drivers (128 cases, 167 controls). However, no information was available on specific engine or vehicle type.

Hayes *et al*. (1989) performed a pooled analysis of data from three U.S. case-control studies carried out by the National Cancer Institute. In this pooled analysis, the authors examined the association between employment in motor exhaust-related occupations and lung cancer risk, adjusting for confounding by smoking and other factors. Subjects for the pooled analysis were restricted to males with an available occupational history (recoded and standardized across the three studies). In total, 2,291 cases of lung cancer and 2,570 controls were eligible. Information examined included all jobs held for six months or more (industry, occupation, and number of

years employed), usual number of cigarettes smoked, birth year, interview type (i.e. with the study subject or the next-of-kin), and study location. All truck drivers employed for longer than 10 years (112 cases, 106 controls) had an OR for lung cancer of 1.5 (95% C.I. = 1.1-2.0) after adjusting for birth cohort, usual daily cigarette use, and state. Subsetting truck drivers into either short-haul (route man/delivery man) or other driver (including long-haul) categories did not substantially alter the associated risk estimates, OR = 1.8 (95% C.I. = 1.0-3.4) for ≥ 10 years as a route man/delivery man versus 1.4 (95% C.I. = 1.0-1.9) for other truck drivers. There was also modest evidence of a trend of increased risk with increasing duration of employment ($p<0.05$), with ORs for <2 years, 2-9 years, 10-19 years, and ≥ 20 years of employment of 1.0 (95% C.I. = 0.7-1.6), 1.0 (95% C.I. = 0.8-1.4), 1.4 (95% C.I. = 0.9-2.2), and 1.5 (95% C.I. = 1.0-2.3), although the OR for only one duration stratum (≥ 20 year) was statistically significant. No specific information was given regarding diesel exposure or engine type.

Steenland *et al*. (1990) performed a case-control study of Teamster Union members who died of lung cancer in 1982-83 (n = 996). Controls included every sixth members' death in the same time period (n = 1,085), excluding deaths from lung and bladder cancer and motor vehicle accidents. Employment data were obtained from two sources -- union work records, which did not specify whether drivers drove diesel or gasoline-powered vehicles, and next-of-kin questionnaires, which did elicit diesel-specific exposure information. However, 90% of the truck drivers identified as diesel truck drivers by next-of-kin were categorized as long-haul drivers by the union. Additionally, the data obtained from next-of-kin included information on several potential confounders (such as smoking, diet and asbestos exposure), which was utilized in all analyses. Analyses were conducted using both sources of work history. No single type of main job category (long- and short-haul truck drivers, gasoline- and diesel-powered trucks, dock workers, or truck mechanics) had a significant increase in risk, although several category estimates were elevated. For example, using union work records resulted in an OR of 1.27 (95% C.I. = 0.83-1.93) for long-haul drivers, while the estimate based on data from next-of-kin resulted in an OR of 1.42 (95% C.I. = 0.89-2.26) for primarily diesel truck drivers. Stratifying the different union job categories by duration of employment gave slightly higher estimates for subgroups with the longest exposures: OR = 1.55 (95% C.I. = 0.97-2.47) for long-haul drivers with 18 or more years exposure and OR = 1.89 (95% C.I. = 1.04-3.42) for diesel truck drivers with greater than 34 years of exposure.

Using cancer registry data, Burns and Swanson (1991) conducted a study of lung cancer by occupation in Detroit. Occupational and smoking histories were obtained by telephone interview for 5,935 lung cancer cases and 3,956 colon or rectal cancer controls diagnosed between 1984 and 1987. After adjusting for smoking, an OR of 2.40 (95% C.I. = 1.65-3.48) was calculated for white male drivers. The smoking- and race-adjusted OR for all drivers (238 cases, 86 controls) was 1.88 (95% C.I. = 1.37-2.58), while drivers of "heavy trucks" (166 cases, 48 controls), maintained a higher risk even after adjustment for smoking, OR = 2.31 (95% C.I. = 1.56-3.42). Mechanics also had a significantly elevated OR for lung cancer (OR = 1.72, 95% C.I. = 1.15-2.59). The types of vehicle engines were not specified.

In a follow-up analysis of the same study population, Swanson *et al*. (1993) presented risk estimates for lung cancer and a number of occupations stratified by years of employment. White male drivers of heavy trucks (237 cases) had smoking-adjusted ORs of 1.4 (95% C.I. = 0.8-2.4) for 1-9 years, 1.6 (95% C.I. = 0.8-3.5) for 10-19 years, and 2.5 (95% C.I. = 1.1-4.4) for 20 or more years (χ^2 test for trend: $p<0.05$). Cases in individuals classified as drivers of light trucks (n = 82) also demonstrated a significant trend of increasing risk with increasing duration of employment, OR = 1.7 (95% C.I. = 0.9-3.3) for 1-9 years and OR = 2.1 (95% C.I. = 0.9-4.6) for 10 or more years. Unlike the comparable stratum for drivers of heavy trucks, the estimate for longest duration stratum, ≥10 years, was not statistically significant.

Rafnsson and Gunnarsdottir (1991) conducted a retrospective cohort study of truck and taxi drivers in Iceland followed from 1951 to 1988. The subjects were selected from the membership rolls of the truck drivers' union. Nearly all trucks were equipped with diesel engines after 1950. Using national mortality rates as an external comparison, the SMR for all truck drivers was 2.14 (95% C.I. = 1.37-3.18) based on 24 observed cases. In an analysis by duration of employment or by period of follow-up, no dose-response relationship was apparent; however, this could be due in part to the small number of cases. Smoking data were not available, but deaths due to respiratory disease were significantly lower than expected (SMR = 0.50 95% C.I. = 0.28-0.82).

Guberan *et al*. (1992) retrospectively studied 1,726 Swiss professional drivers, individuals holding special licenses for driving trucks (n = 1,278), taxis (n = 128) or buses (n = 320), of whom 818 were licensed between 1949 and 1961. The authors estimated that between one-quarter and one-third of the truck drivers engaged in medium- or long-haul driving. No smoking data were available. Cancer mortality for professional drivers from 1949-86 was significantly increased (SMR = 1.50, 95% C.I. = 1.23-1.81) after allowing for 15 years of latency. A significant ($p<0.02$) upward trend in lung cancer mortality with time from first exposure was also observed: SMRs = 0.67, 1.18, 1.30, 1.35, and 2.59 for 0-14, 15-24, 25-34, 35-44, and ≥ 45 years, respectively (no confidence intervals reported).

Hansen (1993) reported on a cohort of 14,225 Danish truck drivers followed for ten years. All subjects were selected from among unskilled laborers identified from 1970 national census files. The comparison group included 43,024 unskilled laborers considered unexposed to vehicle exhaust. There were no data on smoking, but the comparison group was reportedly chosen to resemble the truck drivers with respect to life style, social class, educational background and work-related demands on strength and fitness. With a comparison group of the same social class, one expects similar distributions of cigarette smoking in the exposed and unexposed groups, though there may have been some asymmetry in this instance because more than 1/3 of the comparison group were from rural areas where smoking prevalence tends to be lower. The SMR for truck drivers was 1.60 (95% C.I. = 1.26-2.00) with 76 exposed cases. The report noted that diesel engines have comprised most of the Danish fleet of trucks since the late 1940s.

Pfluger and Minder (1994) analyzed the lung cancer mortality of Swiss professional drivers ("chauffeurs"), which included truck, bus and taxi drivers, whom the authors considered to have had chronic exposure to diesel exhaust. Occupations were determined from death certificates,

while census data were used to compute population-based, age- and occupation-specific death rates. Although individual smoking data were unavailable, an indirect adjustment for smoking-attributable lung cancer mortality was conducted based on occupation-specific smoking rates. Before adjusting for smoking, the SMR for "chauffeurs" was 2.27 (95% C.I. = 1.99-2.58); after accounting for group smoking rates a modest, statistically significant increase remained, SMR 1.48 (95% C.I. = 1.30-1.68).

The studies that have examined the lung cancer risk to truck drivers are summarized in Table 6.5. These studies have consistently reported small increases in lung cancer relative risk. However, the studies suffer from various deficiencies, including small numbers of subjects, inadequate adjustment for confounding, and crude exposure assessments, usually based on occupational classification. Most of the earlier studies did not adjust for smoking. Because of evidence that truck drivers have a higher smoking prevalence (Wynder and Higgins, 1986), individual studies that do not account for smoking generally provide limited evidence regarding carcinogenicity. Before 1988, the two studies that took smoking into account, Williams *et al*. (1977) and Hall & Wynder (1984), had ORs of 1.4 - 1.5, which were not statistically significant. The third study that accounted for smoking (Damber and Larsson, 1985, 1987), only found significantly elevated risks in truck drivers who smoked after stratifying on age (i.e., only for those > 70 years old at diagnosis). However, in the follow-up study, after analyzing for duration of employment (20 or more years), elevated but nonsignificant risks were observed for all professional drivers combined (Damber and Larsson, 1987).

By comparison, the majority of studies published since 1988 have adjusted for smoking to varying degrees. Of the smoking-adjusted population based studies, two of four found statistically significant increases in the relative risk for lung cancer associated with occupation as a truck driver, especially in individuals employed for 10 or more years (Hayes *et al*. 1989; Swanson *et al*. 1993). In addition, both studies reported some evidence of a positive trend between increased duration of employment and risk for lung cancer. Although both found statistically significant trends ($p<0.05$), the only stratum with statistically significant relative risk estimates was that including 20 or more years' employment as a truck driver, with ORs of 1.5 (95% C.I. = 1.0-2.3) and 2.5 (95% C.I. = 1.1-4.4), reported by Hayes *et al*. (1989) and Swanson *et al*. (1993), respectively.

Three of the six more recent industry-specific studies adjusted for smoking, at either the individual (Benhamou *et al*. (1988) and Steenland *et al*. 1990) or group level (Pfluger and Minder 1994). The two studies of professional drivers, a portion of which included truck drivers, found significantly elevated estimates of relative risk with smoking-adjusted ORs of 1.42 (95% C.I. = 1.07-1.89) and 1.48 (95% C.I. = 1.30-1.68) (Benhamou *et al*., 1988 and Pfluger and Minder, 1994, respectively). The one smoking-adjusted study focusing on trucking, Steenland *et al*. (1990), found elevated relative risk estimates for several occupational and duration of employment categories; however, the only statistically significant risk estimate found was for diesel truck drivers with greater than 34 years of exposure, (OR = 1.89; 95% C.I. = 1.04-3.42).

While several population-based studies enrolled a large number of subjects overall (Williams *et al*. 1977; Milne *et al*. 1983; Hall and Wynder, 1984; Damber and Larsson, 1987; Boffetta *et al*.

1988), the actual numbers of subjects occupationally exposed to diesel exhaust (considered here as truck drivers) were small. Of the larger, general population studies (Hayes *et al*. 1989; Benhamou *et al*. 1988; Boffetta *et al*. 1990; Swanson *et al*. 1993) and industry- or occupation-specific studies (Ahlberg *et al*. 1981; Rafnsson and Gunnarsdottir, 1991; Guberan *et al*. 1992; Hansen *et al*. 1993; Pfluger and Minder, 1994; Steenland *et al*. 1990) with greater numbers of truck drivers, significantly elevated smoking-adjusted risk estimates were limited mainly to the case-control studies described above (Hayes *et al*. 1989; Benhamou *et al*. 1988; Steenland *et al*. 1990; Swanson *et al*. 1993; Pfluger and Minder, 1994). Although several industry-specific cohort studies found significantly elevated risks associated with truck or professional driving, with SMRs ranging between 1.33 and 2.14, all lacked smoking data.

6.2.1.2 STUDIES OF LUNG CANCER AMONG TRANSPORT AND EQUIPMENT WORKERS

Raffle (1957) conducted a cohort study of London Transport employees (bus and trolley workers, bus engineers), aged 45 to 64, who were followed between 1950 and 1954. Although the follow-up duration was short, 30 deaths from lung cancer were observed in men aged 55-64. Using other company employees as a comparison group, the SMR was 1.4 (no confidence intervals reported). No data on smoking were available.

Waller (1981) assessed mortality in five categories of London transport employees, including drivers, conductors, engineers, motormen, and guards. This study included some of the data described by Raffle (1957). The SMR for lung cancer, using London males as a comparison population, was 0.79 for the entire cohort. Again, no smoking information was available and, as with the earlier study, only cases arising during employment were considered. Subjects were not followed into retirement or after leaving service, thereby allowing for incomplete ascertainment of cases.

Rushton *et al*. (1983) reported a retrospective cohort study of 8,684 maintenance men with at least one year of service between 1967 and 1975 in London Transport bus garages. The London Transport bus fleet had been fully dieselized since 1950 and these men were expected to have had appreciable exposure to diesel exhaust. Though all-cause mortality compared to the general population was decreased (SMR = 0.84, p<0.000l), lung cancer mortality was not (SMR = l.01, p = 0.94). No smoking data were available, and no analyses based on length of service were presented. As the authors concluded, the study had limited power and the follow-up time was short (six years on average).

Heavy construction equipment workers are potentially exposed to diesel exhaust. For this reason Wong *et al*. (1985) carried out a retrospective mortality study on members of an operating engineers' local union who had at least one year of service between 1964 and 1978 (n = 34,156). Work histories were obtained from job dispatch tapes kept at the union and vital status for separated workers was obtained from the Social Security Administration. Death certificates were obtained for 97% of deceased workers and SMRs were calculated relative to U.S. population mortality rates. For all-cause mortality the SMR was 0.81 (95% C.I. = 0.79-0.84) and for lung cancer the SMR was 0.99 (95% C.I. = 0.88-1.10). Analyses of lung cancer mortality by length of

union membership and by latency showed small positive (but nonsignificant) trends, accompanied by more marked trends for emphysema mortality. An excess of lung cancer was found for retirees. For those who retired only when they reached age 65, the SMR for lung cancer was 1.30 (95% C.I. = 1.04-1.60). In this group there was also a large excess of deaths from emphysema (SMR = 2.75, 95% C.I. = 2.09-3.55) suggesting excess smoking relative to the reference population. Information on smoking habits was not available for this analysis, although a small survey did not show any statistically significant difference between the smoking habits of union members and those of the general population.

Buiatti *et al*. (1985) investigated the occupational factors associated with lung cancer in an Italian hospital-based study during 1981 through 1983. All eligible cases (n = 376) and controls (n = 892) residing in metropolitan Florence were interviewed regarding occupation and smoking status. Adjusted risk estimates (age, residence and smoking) were provided for men in transportation occupations only, with an OR of 1.1 (95% C.I. = 0.7-1.6), based on 45 cases and 99 controls. No specific vehicle type or duration-of-employment analysis was presented.

Edling *et al*. (1987) reported on the mortality experience of a cohort of 694 Swedish bus garage employees followed from 1951 through 1983. Using age-adjusted national rates to calculate expected numbers of cases, the SMR was 0.67 (six observed/nine expected). Five of these six cases occurred in bus drivers versus 7.2 expected (SMR = 0.69). No data were available for smoking and the study power was clearly limited.

In the American Cancer Society prospective mortality study, discussed above in relation to truck drivers (Section 6.2.1.1), Boffetta *et al*. (1988) found a significantly elevated age- and smoking-adjusted RR of 2.60 (95% C.I. = 1.12-6.06) for heavy equipment operators, based on five lung cancer deaths.

Netterström (1988) conducted a death certificate and cancer registry-based study of cancer in 2,465 Danish bus drivers during the period 1978 through 1984. The SMR for lung cancer (unadjusted for smoking) was 0.87 (95% C.I. = 0.48-1.43), based on 15 cases. The mean value for employment duration among the lung cancer cases was 30 years (range = 12 to 39 years).

In their study of motor exhaust-related occupations, Hayes *et al*. (1989) (see Section 6.2.1.1) also examined the risk for heavy equipment operators. After adjusting for age, residence, and smoking, the ORs for operators were elevated but not statistically significant for either duration of employment stratum: OR = 1.5 (95% C.I = 0.4-5.3) for less than 10 years, and OR = 2.1 (95% C.I. = 0.6-7.1) for $\geq$ 10 years of employment. However, there were few operators identified in this study, i.e., 17 cases and 16 controls.

Gustavsson *et al*. (1990) examined lung cancer incidence and mortality in 695 men who had worked in bus garages in Stockholm for at least six months between 1945 and 1970. The subjects were followed until 1986. Exposure was categorized with a metric based on period-specific job tasks and duration of employment. The overall lung cancer SMR (relative to the Stockholm working population) was elevated, but not statistically significant (SMR = 1.22; 95% C.I. = 0.71-1.96). In a more detailed nested case-control analysis of 20 lung cancer cases, the resulting

estimated RRs increased with the diesel-exhaust exposure index: RRs were 1.34 (95% C.I. = 1.09-1.64) for low, 1.81 (95% C.I. = 1.20-2.71) for medium, and 2.43 (95% 1.32-4.47) for high cumulative exposure to diesel exhaust. In this nested case-control analysis, six controls were age matched (± 2 years) to each lung cancer case by selecting noncases randomly from the garage employee cohort at the time of case diagnosis. Precision was limited by the small number of cases. Although the report did not include data on cigarette smoking, the authors suggested that the nested case-control design decreased the potential confounding due to smoking since workers with high and low exposure belonged to the same occupational category and were likely to have had similar smoking habits.

Most studies of transportation workers are limited by small sample size, lack of smoking data, or limited follow-up. None of the three studies of London transportation workers, drivers or garage workers, (Raffle, 1957; Waller, 1981; Rushton *et al*. 1983) obtained information on smoking. In addition, two lacked sufficient follow-up (Raffle, 1957; Rushton *et al*. 1983), excluded retirees, or suffered from small sample size (Raffle, 1957; Waller, 1981). Of the other European studies focusing on bus company employees (Edling *et al*., 1987; Netterström, 1988; Gustavsson *et al*. 1990), only Gustavsson *et al*. (1990) found an elevated risk for lung cancer, with an overall SMR of 1.22 (95% C.I. = 0.71-1.96). However, in the more detailed nested case-control analysis using conditional logistic regression, estimated RRs increased with the cumulative diesel-exhaust exposure index, as noted above.

Of the three studies reporting increased risks for heavy equipment operators (Wong *et al*., 1985; Boffetta *et al*., 1988; Hayes *et al*., 1989), only the RR reported by Boffetta *et al*. (1988) was statistically significant (RR = 2.6; 95% C.I. = 1.12-6.06). However, this estimate was based on only five lung cancer deaths. The large industry-specific cohort study of Wong *et al*. (1985) did not find an elevated risk for lung cancer among unionized heavy equipment operators (SMR = 0.99; 95% C.I. = 0.88-1.10). A subset of individuals retiring at age 65 did have a significantly elevated risk, but a group excess in emphysema deaths (SMR = 2.75; 95% C.I. = 2.09-3.55) and the absence of smoking data suggest that the increased risk may have been related more to tobacco use than to diesel exhaust exposure.

6.2.1.3 STUDY OF LUNG CANCER AMONG DOCK WORKERS

Gustafsson *et al*. (1986) carried out a retrospective cohort study of mortality and cancer incidence in a group of 6,071 Swedish dock workers employed for at least six months before 1974. Workers were followed from 1961, by which time Swedish ports used largely diesel-powered trucks, until 1981. Relative to the Swedish male population, the respiratory cancer mortality was significantly elevated (SMR = 1.29, 95% C.I. = 1.02-1.63). Lung cancer incidence was also elevated (SIR = 1.53, 95% C.I. = 1.24-1.80). No information on smoking habits was available.

In a nested case-control study of Swedish dock workers, Emmelin *et al*. (1993) studied 50 lung cancer cases (from the Gustafsson *et al*. (1986) cohort) and 154 matched controls. The study investigators utilized company records on annual fuel consumption and machine hours, in combination with individual employment histories (job locations and duration) to create three

exposure categories. ORs were estimated for the three exposure categories for both smokers and for nonsmokers. An increasing trend of risk with exposure for both smokers and nonsmokers was reported, but the trend for nonsmokers is not informative because of the small numbers of cases (only 1 to 3 cases per exposure category). For smokers the trend is evident, and the top two exposure categories have significantly elevated risks, even though confidence intervals are wide (see Table 6.5). The smoking-adjusted ORs for the high-exposure groups within the three exposure classifications ("machine time", "fuel consumption", "exposed time") were 2.9 (90% C.I. = 0.6-14.4), 2.9 (90% C.I. = 0.7-11.5) and 6.8 (90% C.I. = 1.3-34.9).

Although the initial report on the entire cohort of Swedish dock workers indicated a significantly elevated risk for lung cancer, the lack of smoking data prevented estimation of a diesel-specific risk (Gustafsson *et al*. 1986). With the availability of smoking and employment histories in the later nested case-control follow-up report of Emmelin *et al*. (1993), several alternative exposure variables were created and assessed, stratifying on smoking status. Although an increasing trend of risk with exposure was identified, only small numbers of exposed cases were included within the classifications. Smoking-adjusted elevated risks were found for all high-exposure groups; however, these were reported as significant at the $p<0.10$ level.

6.2.1.4 STUDIES OF LUNG CANCER AMONG RAILWAY WORKERS

In 1959, Kaplan studied lung cancer mortality among employees of the Baltimore and Ohio Railroad. This railroad initiated locomotive dieselization in 1935, completing this process by 1958. Workers employed at any time between 1953 and 1958 were eligible for entry into the cohort; 154 deaths from primary cancers of the lung or bronchus were identified. Exposure was categorized into three groups by job type. The lung cancer SMR for the most exposed group, relative to the general population, was 0.875. The limited number of years of exposure to diesel exhaust for some members of the cohort and the abbreviated follow-up time do not allow for sufficient latency to be informative regarding the relationship of diesel exhaust exposure to lung cancer. In addition, no data on smoking were available.

In the Third National Cancer Survey discussed above (see Section 6.2.1.1), Williams *et al*. (1977) found a nonsignificant increased risk for railroad workers among lung cancer patients, OR = 1.40, based on 12 cases (no confidence intervals reported).

Howe *et al*. (1983) carried out a mortality study of 43,826 male pensioners of the Canadian National Railroad. The cohort consisted of all male pensioners who were alive at the beginning of 1965. Subjects were followed until 1977, by which time 933 deaths from respiratory cancer (trachea, bronchus and lung) had been recorded. Occupations at the time of retirement were classified as "nonexposed", "possibly exposed" or "probably exposed". Analysis restricted to individuals retiring after 1950 (n = 897 cases) yielded relative risks of 1.00, 1.20 ($p = 0.013$), and 1.35 ($p<0.001$) for the three exposure groups, respectively (test for trend: $p<0.00l$). There was little change in these effect estimates when individuals involved in locomotive maintenance (and who therefore may have been exposed to asbestos) were excluded from the analysis (n = 69).

This study also found coal dust to be associated with lung cancer, with a similar increasing trend with degree of exposure. Because of a high degree of overlap between exposures to coal dust and to diesel exhaust, the authors could not separate the effects of the two. However, since there is evidence from animal and human studies for the carcinogenicity of diesel exhaust, but such evidence does not exist for coal dust, the apparent effect of coal dust was more likely to have been due to confounding by diesel exhaust, rather than vice versa. No smoking information was available for this study, although there were increasing trends with degree of diesel exposure for mortality from emphysema (SMRs = 1.00, 1.35, and 1.44) and other smoking-related cancers combined (SMRs = 1.00, 1.08, and 1.16). The authors suggested that since the results were based on internal comparisons little variation in smoking would be expected among the different diesel exposure groups.

Garshick *et al*. (1987a) carried out a case-control study of lung cancer in U.S. railroad workers. Cases comprised 1,256 lung cancer deaths occurring between 1981 and 1982 in the population of active or retired railroad workers who had had 10 years or more of railroad service and were born in 1900 or later. Two controls who had died of causes other than cancer, suicide or accident were matched to each case by dates of birth and death. Next of kin were interviewed to obtain information about the decedents, including their smoking habits. Job codes were obtained from the Railroad Retirement Board, and an industrial hygiene survey was used to classify the degree of diesel exposure for each job type. Jobs were dichotomously categorized as exposed or not exposed to diesel exhaust.

Garshick *et al*. considered exposure to diesel exhaust to have begun in 1959, since the transition from steam to diesel-powered locomotives took place mainly in the 1950s, and was nearly complete in 1959. Years of diesel exhaust exposure to death or retirement were totaled for each worker. The analysis separated those workers who died at age 65 (retirement age) or older (921 cases and 1,748 controls) from those workers <64 years at death (335 cases and 637 controls). Analysis by logistic regression showed no effect of diesel exhaust in the workers in the older age category, who had substantially less diesel exposure than those in the younger category. For example, 36% of cases and 43% of controls had no exposure in the younger group, while 52% of cases and 53% of controls had no exposure in the older group. Furthermore, 35% of cases and 26% of controls had more than 19 years of diesel exposure in the younger group, while only 3% of cases and controls had more than 19 years of diesel exposure in the older group.

In the group whose members were younger than 64 years old at time of death, the analysis by Garshick *et al*. showed evidence of an exposure-response relationship with an OR of 1.41 (95% C.I. = 1.06-1.88) for 20 or more years of exposure (diesel-years) after adjusting for smoking and asbestos exposure. Excluding exposure occurring within five years of death, the OR for 15 or more years of cumulative diesel exposure was 1.43 (95% C.I. = 1.06-1.94). For workers with 5 to 14 years of cumulative exposure, the OR was 1.07 (95% C.I. = 0.69-1.66) relative to a reference category of 0 to 4 diesel exposure years.

Garshick *et al*. (1988) also conducted a retrospective cohort study of U.S. railroad workers. Eligible for inclusion in the cohort were white males aged 40 to 64 years, who started work between 1939 and 1949 and were still employed in 1959 in designated jobs. Follow-up extended

through 1980. Jobs with recognized asbestos exposure were not included in the job codes selected for study, although some of the selected occupations had at least some potential for asbestos exposure. The cohort consisted of 55,407 men, among whom there were 19,396 deaths, including 1,694 attributable to lung cancer. Diesel exhaust exposure was characterized based on their 1959 job group. Career paths were found to be very stable in the railways, such that a worker aged 40-44 with a diesel-exposed job in 1959 was likely to have a diesel-exposed job 20 years later; similarly a nonexposed person in 1959 was likely to have a nonexposed job 20 years later.

The youngest workers in 1959 had the longest potential duration of diesel exposure in the cohort. In a proportional-hazards model these workers had the highest estimated relative risks for lung cancer associated with diesel exhaust exposure: the relative risk for the group aged 40-44 in 1959 was 1.45 (95% C.I. = 1.11-1.89); for the group aged 45-49 the relative risk was 1.33 (95% C.I. = 1.03-1.73); for the group aged 50-54, 1.12 (95% C.I. = 0.88-1.42); for the group aged 55-59, 1.18 (95% C.I. = 0.94-1.50); and for the group aged 60-64, 0.99 (95% C.I. = 0.74-1.33). Though the results were statistically significant only for the two youngest groups, there was a decreasing trend with increasing age in 1959 (except for the 55-59 year age group), implying declining risk with decreasing duration of exposure.

When exposure to diesel over the last five years before death was excluded, a relationship was apparent between lung cancer risk and duration of exposure. The group with greater than 15 years of cumulative exposure had a RR for lung cancer of 1.72 (95% C.I. = 1.27-2.33); for those with 10 to 14 years of exposure the RR was 1.32 (95% C.I. = 1.13-1.56); for 5 to 9 years, 1.24 (95% C.I. = 1.06-1.44); and for 1-4 years, 1.20 (95% C.I. = 1.01-1.44). All of these results are statistically significant.

Although no smoking information was available for the cohort, the previous case-control study of railway workers by the same group (Garshick *et al*., 1987a) reported that little change occurred in the estimates of diesel exhaust effect due to adjustment for smoking habits and asbestos exposure (unadjusted OR = 1.39, 95% C.I. = 1.05-1.83; adjusted OR = 1.41, 95% C.I. = 1.06-1.88). In this analysis, the larger percentage of workers whose pack-year history was unknown (23% of cases and 22% of controls) was treated as a separate category of smoking. In additional analyses using only those workers for whom the investigators had detailed smoking data (n = 758), the ORs for 20 yr of diesel exposure ranged from 1.50-1.53, adjusted for asbestos exposure and several specifications of cigarette smoking history. These models included pack-years as a single continuous variable, as two independent variables (cigarettes per day and years of smoking), or as a categorical variable classified in terms of the number of years the study subject had stopped smoking prior to death. These analyses suggested that the diesel exhaust-lung cancer odds ratios were not confounded by cigarette smoking in this population. Moreover, in a group of railroad workers previously surveyed for asbestos exposure (Garshick *et al*., 1987b) there was no difference in smoking prevalence between workers with and without diesel exhaust exposure (data not presented).

It should be noted that the case-control and the cohort studies by Garshick *et al*. involved different study populations: The case-control study (Garshick *et al*. 1987a) contained cases and

controls who had died in 1981 and 1982, whereas the cohort study (Garshick *et al*., 1988) involved deaths occurring up to 1980. Thus, they may be considered different tests of the hypothesis of an association between lung cancer and diesel exhaust exposure, although this does not exclude the possibility of a common bias shared by the two studies, such as exposure to chemicals transported by rail or to suspended dusts and debris.

In the American Cancer Society prospective mortality study mentioned above (see Section 6.2.1.1), Boffetta *et al*. (1988) found an age- and smoking-adjusted RR of 1.59 (95% C.I. = 0.94-2.69) for lung cancer mortality in railroad workers. This estimate was based on only 14 lung cancer deaths.

Swanson *et al*., (1993, as discussed in Section 6.2.1.1) also examined the industrial category of railroad workers in their case-control study of lung cancer. The smoking-adjusted odds ratios for white males (67 cases) were 1.2 (95% C.I. = 0.5-2.7) for 1-9 years of employment and 2.4 (95% C.I. = 1.1-5.1) for more than 10 years of employment (χ^2 test for trend: $p<0.05$). Elevated, but nonsignificant, smoking-adjusted ORs were also associated with the 31 lung cancer cases occurring in African-American railroad workers, OR = 2.6 (95% C.I. = 0.8-7.9) for 1-9 years and OR = 2.7 for $\geq$ 10 years of employment (95% C.I. = 0.6-12.1).

Nokso-Koivisto and Pukkala (1994) compared the incidence of lung cancer among locomotive drivers to the total Finnish population. The retrospective cohort consisted of the 8,391 members of the Finnish Locomotive Drivers' Association from 1953 until 1991 (retired drivers remain members until death). After excluding 302 members for lack of personal identification information, an overall standardized incidence ratio (SIR) of 0.86 (95% C.I. = 0.75-0.97) was found (236 cases). The overall incidence for all cancer sites was also lower than expected, SIR 0.95 (95% C.I. = 0.89-1.01) but the incidence of mesothelioma (SIR 4.05, 95% C.I. = 1.75-7.97) and oral cavity/pharyngeal cancers (SIR 1.75, 95% C.I. = 1.02-2.80) were significantly increased. Prior to the 1970s Finnish drivers trained for 2 years in railroad workshops, where significant exposure to asbestos occurred routinely during steam engine maintenance, with little, if any, diesel exposure. Only drivers working after this period had the potential for substantial exposure to diesel exhaust, and the electrification of the railroad in the 1970s and 1980s may also have reduced the proportion of the cohort's person-years that truly reflect exposure to diesel exhaust. No data on smoking within the cohort were available, though a cross-sectional study of locomotive drivers in 1976 showed that the prevalences of current smokers (40%), ex-smokers (34%), and never-smokers (26%) were similar to those in the Finnish population as a whole.

All three population-based case-control studies found elevated risks for lung cancer in railroad workers (Williams *et al*., 1977; Boffetta *et al*., 1988; Swanson *et al*., 1993); however, only the study by Swanson *et al*. (1993) found a statistically significant increase, with a smoking-adjusted OR of 2.4 (95% C.I. = 1.1-5.1) for workers with ten or more years of employment. This study also found evidence of a significant exposure-response relationship for the 67 cases observed in white railroad workers. Williams *et al*. (1977) and Boffetta *et al*. (1988) had relatively fewer railroad workers (12 and 14 cases respectively) and no information on duration of exposure.

In the railroad industry-based studies, three of the larger studies identified statistically significant increases in relative risk (Howe *et al*., 1983; Garshick *et al*., 1987a; Garshick *et al*., 1988). The large cohort reported on by Howe *et al*. (1983) found elevated risks for individuals categorized as "probably" and "possibly" exposed to diesel exhaust, but without adjustment for smoking or duration of employment, the underlying risk is uncertain. In both the case-control and cohort studies by Garshick *et al*., 1987a, 1988), significantly increased risks were associated with long-term employment in diesel-related railroad jobs. Both studies had substantial exposure assessment, sufficient latency, and duration of employment data, and the case-control investigation also controlled for potential confounding by smoking and by asbestos exposure. In contrast, the study by Nokso-Koivisto *et al*. (1994), found no increase in lung cancer risk among Finnish locomotive engineers, though the description of the cohort indicates the earlier cases were unlikely to have experienced any diesel exposure.

6.2.1.5 STUDIES OF ANY DIESEL EXHAUST EXPOSURE

Several occupational mortality studies investigating the association between lung cancer risk and occupation have indirectly linked either diesel exhaust or diesel-related occupations to increased cancer risk. However, many of these studies are small and therefore have low statistical power. For example, in a case-control registry study of oat cell carcinoma, Wegman and Peters (1978) found an increase in risk associated with employment as a transportation equipment operative (nine cases). Smoking and occupational histories were obtained by next-of-kin interview; however, smoking-adjusted risk was not estimated since almost all cases and controls smoked. In another registry study in New Mexico, Lerchen *et al*. (1987) obtained personal interviews with 506 patients and 771 controls, both males and females. The number of cases of those reporting an occupation with diesel exhaust was seven, which was also too small for significant inference.

A death-certificate-based study by Coggon *et al*. (1984) on all males in England and Wales under age 40 dying of lung cancer between 1975 and 1979 found elevated risks for all diesel exhaust-related occupations (OR = 1.3; 95% C.I. = 1.0-1.6). A job-exposure matrix was developed based on occupations as listed on the death certificates -- within the matrix, exposures were graded as high, low or none. Of the 172 cases assigned to diesel-exposed occupations, 32 were considered to have had high exposure, compared to 57 controls, for an OR of 1.1 (95% C.I. = 0.7-1.8). In a similar, larger death certificate study, Magnani *et al*. (1988) investigated 31,925 lung cancers in British men ages 15-64 during 1970-72. For all diesel exhaust exposure grades combined, the SMR was 1.07 (95% C.I. = 1.04-1.10). Adjusting for social class decreased the SMR to 0.97 (95% C.I. = 0.94-0.99). No data on smoking were available in either of these studies.

Siemiatycki *et al*. (1988) conducted a case-referent study of 3,726 Canadian cancer patients, with a response rate of 82%. The authors found that diesel exhaust exposure was associated with squamous cell carcinoma of the lung (n = 81) (OR = 1.2; 90% C.I. = 1.0-1.5). When these cases were stratified further by high- and low-exposure categories together with longer and shorter duration, there were no statistically significant results. Reported results were adjusted for smoking, age, socioeconomic status, ethnic group, and blue/white collar job history.

Only a few studies have been reported from other industries in which workers are potentially exposed to diesel exhaust. Bender *et al*. (1989) conducted an occupational cohort mortality investigation of Minnesota highway maintenance workers. Among the 4,849 eligible men employed (for at least one day) between 1945 and 1984, a significantly decreased risk of death due to lung cancer was observed (SMR = 0.69, 95% C.I. = 0.52-0.90). The cohort also had significantly lower than expected mortality from all causes (SMR = 0.91, 95% C.I. = 0.86-0.96) and from all cancers (SMR = 0.83, 95% C.I. = 0.73-0.94). Information on smoking was not available.

Kauppinen *et al*. (1993) investigated engine exhaust as one of several chemicals woodworkers were exposed to in Finland. In a nested case-control study of a previous woodworking cohort, 136 cases diagnosed between 1957 and 1982 were matched to 408 controls. An elevated risk was found for engine exhaust (exposures to diesel exhaust could not be segregated from those to gasoline) with a smoking-adjusted OR for any exposure of 1.70 (90% C.I. = 0.55-5.20), which increased in the subset exposed for more than five years (OR = 2.21, 90% C.I. = 0.65-7.48). However, the overall number of cases with potential diesel exposure was small (n = 11).

6.2.2 META-ANALYSIS ON THE RELATIONSHIP BETWEEN OCCUPATIONAL EXPOSURE TO DIESEL EXHAUST AND LUNG CANCER

A meta-analysis was conducted to summarize and help interpret the published reports examining the relationship of lung cancer and exposure to diesel exhaust (See Appendix C). A meta-analysis systematically combines the results of previous studies in order to generate a quantitative summary of a body of research and to examine the sources of variability among studies (for review see Petitti, 1994). The variability, or heterogeneity, of results among studies may exist due to numerous factors, including differences in study design, exposures experienced by study subjects, methods and accuracy of exposure ascertainment, length of follow-up, and control of confounders (such as smoking).

As described in Appendix C, 30 studies, contributing a total of 39 effect estimates, were utilized in the meta-analysis. The pooled relative risks for lung cancer from all 39 risk estimates combined varied with the statistical model used, 1.04 (95% C.I. = 1.02-1.06) under the fixed-effects model and 1.33 (95% C.I. = 1.21-1.46) with the random-effects model. However, significant evidence of heterogeneity was found (DerSimonian and Laird Q-statistic = 214.59, 38 d.f., $p<0.001$]). Heterogeneity in this context refers to large between-study variability. The presence of heterogeneity undermines the validity of the pooled estimates, and suggests the need for additional analysis to identify the sources of heterogeneity. As discussed in detail in Appendix C, this involved deriving pooled estimates for a variety of subsets of the reports.

Through subset analysis, several factors were identified which strongly influenced both the magnitude and the degree of heterogeneity of the pooled risk estimates: (1) whether or not a study adjusted for smoking, (2) study design (3) the exposure assessment, as developed from occupational categories, (4) the presence of selection bias, as manifested by an observed “healthy worker effect”, and other study characteristics (See Appendix C). By stratifying the meta-analysis on whether the risk estimates accounted for smoking, the effect of failure to control for

this exposure on the pooled estimate became readily apparent. Not only did the positive association between diesel-exhaust exposure and lung cancer persist, but the pooled risk estimate increased to 1.43 (95% C.I. = 1.31-1.57, random-effects model) with little evidence of heterogeneity among the 12 studies controlling for smoking.

The case-control studies (15 included in the meta-analysis) gave a summary estimate of 1.44 (95% C.I. = 1.33-1.56), again with little evidence of heterogeneity, while the estimate based on the results of the cohort studies remained heterogeneous. The lower pooled RR estimate and substantial heterogeneity obtained from the cohort subanalysis was probably due at least in part to failure to adjust for smoking, as only one of sixteen cohort studies controlled for this confounder, while most case-control studies did (11 of 14 studies, accounting for 17 of the 20 case-control risk estimates).

The "healthy worker effect" (HWE - here based on significantly lower than expected all-cause mortality) is a manifestation of selection bias related to hiring and retention of workers who are typically healthier than the general population, resulting in spuriously lower risk estimates for a variety of illnesses, including those potentially related to occupational exposures. Subsetting the cohort studies into those with and those without an obvious healthy worker effect markedly reduced the degree of heterogeneity in the group without the HWE (Q-statistic = 11.190, 9 d.f., p = 0.27), and produced an increase in the magnitude of the pooled relative risk (RR = 1.52, 95% C.I. = 1.36-1.71-1.78, random-effects model). In contrast, those studies whose results were characterized by the presence of a HWE continued to show substantial heterogeneity, and the pooled risk estimates declined. Thus, selection bias is likely to have played a role in the heterogeneity observed among the cohort studies. Selection bias results from choosing a study sample that is not representative of the entire population that could have been studied, and can distort the measure of effect (e.g., relative risk or odds ratio) (Rothman 1986).

With respect to exposure assessment, statistically significant pooled estimates of elevated risk lacking evidence of heterogeneity were identified in several occupational subgroup analyses, both with and without additional stratification for smoking. Prior to stratifying by adjustment for smoking, the occupational subgroups involving trucking (pooled RR = 1.47, 95% C.I. = 1.33-1.63), the railroad industry (random-effects pooled RR = 1.45, 95% C.I. = 1.08-1.93), mechanics and garage workers (random-effects pooled RR = 1.35 (95% C.I. = 1.03-1.78), general transportation and professional drivers (random-effects pooled RR = 1.45, 95% C.I. = 1.31-1.60) gave risk estimates greater than the overall pooled risk estimate. The pooled RR estimates for trucking and general transportation and professional drivers showed little to no evidence of heterogeneity; however, estimates for the railroad industry demonstrated considerable heterogeneity (Q statistic = 30.90, $p<0.001$).

Further stratification of the occupational subgroup analysis by adjustment for smoking produced a large impact on the pooled risk estimates, with all smoking-adjusted subgroup estimates displaying little evidence of heterogeneity and leading to increased risk estimates in all but one of the occupational categories. Pooled risk estimates by occupation in smoking-adjusted studies showed little evidence of heterogeneity for several occupations under both models, including truck drivers (random-effects pooled RR = 1.53, 95% C.I. = 1.20-1.94), railroad workers

(random-effects pooled RR = 1.68, 95% C.I. = 1.28-2.19), and diesel mechanics and garage workers (random-effects pooled RR = 1.25, 95% C.I. = 0.87-1.80). The pooled estimates for the heavy equipment operators and dock workers and for the railroad industry studies adjusting for smoking displayed the most dramatic changes relative to the occupational analysis without smoking stratification. Among the former subgroup, the pooled risk estimate changed from 1.28 (random-effects model, 95% C.I. = 0.99-1.66) to 2.43 (95% C.I. = 1.21-4.88). Among the railroad industry studies, the pooled risk estimate also increased substantially (from 1.45 to 1.68, 95% C.I. = 1.28-2.19). In both subgroups, the pooled smoking-adjusted estimates showed little evidence of heterogeneity, though these estimates were based on two studies in the former instance and three in the latter. However, the other two heavy equipment operator and dock worker studies and the other three railroad industry studies that were not adjusted for smoking still displayed evidence of heterogeneity (Q-statistics = 2.933, 1 d.f., p =0.09, and 21.517, 2 d.f., $p<0.001$, respectively).

The meta-analysis also identified evidence of exposure-response relationships in the subgroup analyses based on duration of employment. However, as noted in Appendix C, this analysis was hampered by the absence of duration-specific risk estimates in approximately one-half the studies. While the initial analysis conducted on all the included studies resulted in elevated pooled risk estimates for strata in which exposure durations were greater than 10 years relative to those with less than 10 years of exposure or for which the exposure durations were not clear from the published reports, there was still significant evidence of heterogeneity for several of the duration strata. In contrast, the analysis utilizing only estimates from the smoking-adjusted studies showed some evidence of an exposure-response gradient without evidence of statistical heterogeneity. The summary risks for all three exposure-duration strata were: RR = 1.39 (95% C.I. 1.19-1.63) for < 10 years (based on three estimates from two studies), RR = 1.64 (95% C.I. = 1.40-1.93) for $10 \leq$ to < 20 years (11 estimates from 6 studies), and RR = 1.64 (95% C.I. = 1.26-2.14) for ≥ 20 years (four estimates from four studies). The pooled risk estimate for those studies for which the exposure duration was not clear in the published reports was 1.24 (95% C.I. = 1.00-1.54) (six estimates from four studies) (see Table C-4 in Appendix C).

These results were robust to a variety of sensitivity analyses. In an analysis of potential publication bias, however, there appeared to be a modest increase in the RR estimates with increasing sample size (reflected in a decreased standard error of the estimates). Publication bias, or the increased likelihood or preference for the publication of statistically significant results compared to nonsignificant or null results, may potentially distort pooled risk estimates. Publication bias is generally attributed to journal editorial policies that prefer “positive” results, so that small, statistically nonsignificant studies are less likely to be published than large, statistically nonsignificant studies (Greenland, 1994). However, it should be noted that the studies with the smallest standard errors were almost exclusively cohort studies that did not adjust for smoking and which also had a clear HWE, suggesting that other significant biases are likely to have played a role in creating an appearance of publication bias. Therefore, although publication bias cannot be ruled out, the inclusion of numerous studies of varying sample sizes and statistically insignificant findings, as well as the uncontrolled confounding and likely selection bias affecting many of the larger cohort studies, make it unlikely that the result of this meta-analysis can be completely explained by publication bias.

In summary, the meta-analysis indicated a consistent positive association between occupations involving diesel exhaust exposure and the development of lung cancer. Although substantial heterogeneity existed in the initial pooled analysis, stratification on several factors identified a persistent positive relationship. The major sources of heterogeneity included: (1) whether or not a study adjusted for smoking, (2) study design (3) the exposure assessment, as developed from occupational categories, (4) and the presence of selection bias, as manifested by an observed healthy worker effect. Taking these factors into account tended to increase the estimates of relative risks of lung cancer from occupational exposure to diesel exhaust.

Another independently conducted meta-analysis of diesel exhaust exposure and lung cancer produced remarkably similar results, with an overall pooled relative risk estimate of 1.33 (95% C.I. = 1.24-1.44) (Bhatia *et al*., 1998). In that analysis, the study inclusion and exclusion criteria were somewhat different than those used by OEHHA staff, so that 23 studies were included. Consequently, the results of some of their subset analyses differed from those described in Appendix C. In addition, those authors used only a fixed-effects model to derive pooled risk estimates, and did not focus on explorations of sources of heterogeneity. Nevertheless, Bhatia and co-workers also found a persistent positive relationship between diesel exhaust exposure and lung cancer that could not be attributed to potential confounding by cigarette smoking. Moreover, in the narrower group of studies in their report, they identified a positive exposure-response relationship in studies stratified by exposure duration.

6.2.3 REVIEW OF BLADDER CANCER STUDIES

A number of studies have considered the possibility of a link between diesel exhaust exposure and bladder cancer. Such a link is plausible, given the fact that cigarette smoking is a well-established cause of bladder cancer, and tobacco smoke and diesel exhaust both contain recognized carcinogenic constituents.

A population-based study of bladder cancer drew 480 male and 152 female matched case-control pairs from three Canadian provinces (Howe *et al*., 1980). The relative risk estimate for self-reported exposure to either diesel or traffic fumes was 2.8 (95% C.I. = 0.8-11.8) based on 11 cases compared to 4 controls. An elevated relative risk estimate was also found for railroad work, 9.0 (95% C.I. = 1.2-394.5), based on 9 cases. Although smoking data were collected by the investigators, smoking-adjusted RRs were not reported for these specific subgroups.

In the retrospective cohort study of 43,826 male pensioners of the Canadian National Railway Company described above (section 6.2.1.4), Howe *et al*. (1983) found no excess of deaths due to bladder cancer. Of the 17,838 deaths occurring among the retired railroad workers between 1965 and 1977, 175 were due to bladder cancer compared to the 170 expected from the national Canadian death rates (SMR = 1.03; 90% C.I. = 0.91-1.17).

In a population-based case-control study employing 303 white male cases and 296 controls, Silverman *et al*. (1982) examined the relationship between occupation and bladder cancer in Detroit. The Detroit area has had one of the highest rates of white male bladder cancer mortality in the United States. Data on smoking habits were available. Of the 56 occupations for which

ORs were calculated, having ever been employed as a "truck driver" (42 cases and 18 controls) was the only one for which the lower 95% confidence limit exceeded unity (smoking unadjusted OR = 2.5 [95% C.I. = 1.4-4.4] and adjusted OR = 2.1 [no C.I. presented]). In a smaller group of subjects reporting truck driving as a "usual" occupation (11 cases and 2 controls), a higher smoking-adjusted risk was observed (OR = 5.4, [no C.I. presented]). An exposure-response relationship between increasing duration of employment as a truck driver and risk for bladder cancer was also found (<10 years: OR = 1.4, [no C.I. reported], for 23 cases and 15 controls; ≥ 10 years: OR = 5.5 [95% C.I. = 1.8-17.3], for 16 cases and 3 controls with 3 cases excluded due to lack of duration data; χ^2 test for trend: p = 0.004). The association was greatest for employment as a truck driver after 1950 (OR = 6.5 with 7 cases and 1 control), while risks for earlier decades were lower, and no consistent trend prior to 1950 was observed. Wynder *et al*. (1985) have suggested that, because truck drivers spend a large proportion of their time on the road, they may have been under-represented among the controls, and this may have accounted for the observed associations.

Subsequent to the finding that truck driving was associated with an elevated risk for bladder cancer, Silverman *et al*. (1982) re-interviewed cases (36 of 42) and controls (16 of 18) who had indicated in their initial interview that they had been truck drivers. After asking more diesel-specific questions, thirteen cases and one control reported operating vehicles with diesel exhaust, resulting in a smoking-adjusted RR for "diesel-exposed" truck drivers of 11.9 (95% C.I = 2.3-61.1), when the unexposed group consisted of those who had never been employed as truck drivers. However, only a minority of the cases (n = 5) had actually driven diesel trucks. The majority of the self-reported diesel exposure occurred while operating other vehicles (i.e. mechanic, engineer, deliveryman, bus driver, and end loader).

To test the hypothesis of an association between diesel exhaust exposure and bladder cancer raised in their Detroit study, Silverman *et al*. (1986) analyzed data for 1,909 white male bladder cancer cases and 3,569 controls drawn from all ten centers in the National Bladder Cancer Study using cancer registries throughout the United States. The study identified 488 cases and 742 controls who had ever had employment as a truck driver or deliveryman, giving an age- and smoking-adjusted relative risk for bladder cancer of 1.3 (95% C.I. = 1.1-1.4). The 99 cases and 123 controls who reported truck driver or deliveryman as their usual employment had a RR of 1.5 (95% C.I. = 1.1-2.0). Increasing duration of employment as a truck driver or deliveryman correlated significantly with increasing risk. For <5, 5-9, 10-14, 15-24 and 25+ years, the RRs (with numbers of cases and controls, 7 cases and 10 controls with unknown smoking history and/or duration of employment excluded) were 1.1 (208,379), 1.3 (102,148), 1.7 (58,65), 2.2 (59,52) and 1.1 (54,88), respectively (test for trend: p<0.001). Although in this analysis the longest duration of employment (25+ years) was not associated with increased risk, a monotonic increase in RR for increasing employment duration was found when the analysis was restricted to those who had been first employed as a truck driver at least 50 years prior to diagnosis.

Wynder *et al*. (1985) carried out a hospital-based bladder cancer case-control study using 194 male cases and 582 controls. Classification of exposure to diesel exhaust was based upon self-reported usual occupation. The age- and smoking-adjusted OR for usual employment in an

occupation with exposure to diesel exhaust was 0.87 (95% C.I. = 0.47-1.58) with 16 cases and 50 controls classified as exposed. The OR for usual employment as a bus or truck driver was 0.90 (95% C.I. = 0.44-1.87) with 10 cases and 33 controls, and for employment as a railroad worker 2.0 (95% C.I. = 0.34-11.61) with 2 cases and 3 controls. This study lacked detailed employment histories and contained only small numbers of cases and controls usually employed in occupations associated with diesel exposure.

Another hospital-based study by Iyer *et al*. (1990), involving 136 cases and 272 controls, found a weak association between bladder cancer and any diesel exhaust exposure, whether based on occupation (n = 32) or self-reported diesel exhaust exposure (n = 9), with a smoking-adjusted OR of 1.06 (95% C.I. = 0.64-1.76). Analysis by occupation based on "possible" (19 cases) or "probable" (13 cases including 4 truck or delivery drivers) exposure also found no significant associations, with smoking-adjusted ORs of 1.11 (95% C.I. = 0.60-2.08) and 0.86 (95% C.I. = 0.41-1.81), respectively.

In a hospital-based case-control study in Argentina, Iscovich *et al*. (1987) investigated the reasons for a high incidence of bladder cancer. The 117 cases, paired to both 117 neighborhood and 117 hospital controls, were interviewed regarding smoking and drinking habits and occupational exposures. Although the major risk factors were found to be cigarette smoking and coffee drinking, the combined category of truck and railway drivers showed a significant association with bladder tumors. With 20 cases, 5 hospital and 4 neighborhood controls, the odds ratio for the two occupations combined was 4.31 ($p<0.005$). All truck driver cases were cigarette smokers, while the majority of controls either did not smoke or smoked considerably less (< half-pack per day), making it difficult to separate the effects of smoking from occupational exposures. The report does not mention the extent to which subjects in these two occupations were exposed to diesel exhaust.

A Canadian case-control study of occupation and bladder cancer included 826 cases diagnosed in the provinces of Alberta and Ontario during 1979-82, matched to 792 randomly selected population controls (Risch *et al*. 1988). Only 67% of eligible cases and 53% of eligible controls were interviewed. The investigators found a significantly increased risk for the 309 men ever having had jobs involving contact with traffic or diesel fumes, after adjusting for cumulative pack-years of smoking (OR = 1.53, 95% C.I. = 1.17-2.00). A slightly higher increase in risk was found for the subset (approximately half) employed for at least six months in the period 8 to 28 years prior to diagnosis (i.e., with a minimum latency period of eight years) (OR = 1.69, 95% C.I. = 1.24-2.31).

In a population-based case-control study of 256 male urothelial cancer cases diagnosed in 1985-87 in Stockholm, Steineck *et al*. (1990) found a RR estimate of 1.7 (95% C.I. = 0.9-3.3) associated with diesel exposure, after adjustment for smoking and birth year. Further classifying each subject's exposure as low, moderate or high, and accounting for duration and intensity of exposure, failed to identify a significant association with diesel exhaust (moderate and high exposure RR = 1.1, 95% C.I. = 0.3-4.3).

6.2.4 CAUSAL INFERENCE FOR DIESEL EXHAUST EXPOSURE AND LUNG CANCER

The results of the epidemiological analyses imply that occupational exposure to diesel exhaust is associated with an increased risk of developing lung cancer. While some recent reviews have come to similar conclusions (U.S. EPA, 1994, Health Effects Institute, 1995, World Health Organization, 1996, Boffetta *et al*., 1997), others have not (Stöber and Abel, 1996; Muscat and Wynder, 1995; Morgan *et al*. 1997). The evidence for an association between diesel exhaust exposure and bladder cancer is considerably weaker. This section deals with the evidence for causality in the association between diesel exhaust and cancer of the lung. The following criteria for causal inference are considered: (1) the consistency of the findings; (2) the strength of the associations; (3) the possibility that findings are due to bias; (4) the likelihood that findings are due to chance; (5) evidence for exposure-response relationships; (6) temporality of the associations; and (7) biological plausibility of a causal association.

(1) The consistency of the findings. As summarized in Table 6.5, there is a considerable degree of consistency in finding elevated, although not always statistically significant, lung cancer risks in workers potentially exposed to diesel exhaust within several industries. General population-based case-control studies identified statistically significant increases in lung cancer risk for truck drivers (Hayes *et al*. 1989; Swanson *et al*. 1993), railroad workers (Swanson *et al*., 1993), heavy equipment operators (Boffetta *et al*., 1988), and for self-reported diesel exhaust exposure in general (Siemiatycki *et al*., 1988). All of these statistically elevated estimates were adjusted for smoking. Industry-specific studies, both of case-control and cohort design, identified statistically elevated lung cancer risk for truck drivers (Ahlberg *et al*., 1981; Rafnsson and Gunnarsdottir, 1991; Guberan *et al*., 1992; Hansen *et al*, 1993), professional drivers (Benhamou *et al*., 1988; Pfluger and Minder, 1994) and railroad workers (Howe *et al*., 1983; Garshick *et al*., 1987a, 1988), with a minority of these studies adjusting for smoking (Benhamou *et al*., 1988; Pfluger and Minder, 1994; Garshick *et al*., 1987a).

In order to quantitatively summarize the overall and occupation-specific risks from the body of studies, a meta-analysis was conducted (see Appendix C). The results of this meta-analysis indicate a consistent positive association between occupations involving diesel exhaust exposure and the development of lung cancer. Figure 6.2.1 presents the summary risk estimates and 95% confidence intervals for the major occupational categories, and for the subgroups of studies stratified on adjustment for smoking, obtained from the meta-analysis. Although substantial heterogeneity existed in the initial pooled analysis, stratification on several factors identified a relationship that persisted throughout various influence and sensitivity analyses. Major sources of heterogeneity included adjustment for smoking, exposure assessment as assigned through occupational categories, and manifestations of selection biases such as the healthy worker effect (HWE). Another recently published meta-analysis of diesel exhaust exposure and lung cancer found a similar consistency supportive of a causal relationship (Bhatia *et al*., 1998).

(2) The strength of the associations. The relative risk (RR) estimates obtained in these studies are generally low, with most estimates less than two. RR estimates of this magnitude potentially weaken the evidence of causality, due to the possibility of uncontrolled confounding or other

sources of bias producing the findings. However, RR estimates in this range are found in many areas of medicine and, in conjunction with other components of causal inference, constitute the basis for a variety of clinical and public health interventions to prevent or ameliorate disease. To place the diesel-lung cancer associations in context, the following table represents estimates of relative risk of death from cardiovascular disease in several prospective epidemiological studies:

Study	Estimate of RR
British doctors	1.6
Males in 25 states	
ages 45-64	2.08
ages 65-79	1.36
U.S. Veterans	1.74
Japanese study	1.96
Canadian veterans	1.6
Males in nine states	1.70
Swedish males	1.7
Swedish females	1.3
California occupations	2.0

Source: U.S. Department of Health and Human Services (1989), p.39.

Despite the relatively low magnitude of these estimates, active cigarette smoking is widely recognized as one of the principal causes of heart disease. Several years ago, the Centers for Disease Control estimated that 156,000 deaths/year were due to heart disease caused by cigarette smoking. Similarly, several large national reviews of the evidence have concluded that environmental tobacco smoke (ETS) exposure causes lung cancer in nonsmokers, even though most pooled RRs are in the range of about 1.2 to 1.9 (National Research Council, 1986; U.S. Department of Health and Human Services 1986; National Institutes of Health 1993). In other words, "weak associations" in epidemiology can and have been used repeatedly as a basis for causal inference.

The vast majority of studies reviewed here indicated a positive association between lung cancer and occupational exposure to diesel exhaust. While many of the studies presented multiple estimates of relative risk, 23 of the 40 studies summarized in Table 6-5 contained at least one estimate described as statistically significant or as having a confidence interval whose lower bound exceeded unity. Several studies which accounted for at least one of the two principal confounders, smoking and exposure to asbestos, found significantly elevated risks, especially after longer-term exposures (Garshick *et al.*, 1987a; Garshick *et al*. 1988; Gustavsson *et al*., 1990; Hayes *et al*., 1989; Swanson *et al*., 1993). Additionally, the quantitative summary provided by the meta-analysis demonstrated not only that the increases in lung cancer risk remained after stratification by smoking or occupation, but in several instances increased. For

example, the pooled relative risk for railroad workers increased from 1.45 to 1.68 (95% C.I. = 1.28-2.19) when only smoking-adjusted risk estimates were considered.

(3) The possibility that findings are due to bias. In evaluating these results, one needs to consider confounding, information bias and selection bias. The two most likely confounding exposures are cigarette smoking and asbestos exposure. The importance of controlling for cigarette smoking is demonstrated clearly in the meta-analysis, in which this was a major factor contributing to the heterogeneity among studies. Among those studies that did adjust for this confounder, there remained statistically elevated risks of lung cancer, regardless of occupation. By stratifying the risk estimates in the meta-analysis on whether the studies adjusted for cigarette smoking, the effect of failure to control for this exposure on the pooled estimates became readily apparent. Not only did the positive association between diesel-exhaust exposure and lung cancer persist, but the pooled risk estimate increased to 1.43 (95% C.I. = 1.31-1.57), with little evidence of heterogeneity among the smoking-controlled risk estimates. The meta-analysis of Bhatia *et al*. (1998) and the review of epidemiological studies by the Health Effects Institute (1995) also found that the increased relative risks of lung cancer were unlikely to be explicable by uncontrolled confounding by cigarette smoking. Others have asserted a contrary view (Stöber 1996, McClellan 1989). It has been asserted that control of confounding by smoking that involves dichotomous classification of smoking status or even the use of pack-years as an indicator of exposure are both inadequate and will allow for residual confounding (Muscat 1996). Because smoking is the dominant risk factor for lung cancer, misclassification of this exposure could result in residual confounding. Since much of the information about the study subjects' cigarette smoking was obtained from proxy respondents (primarily spouses), some misclassification probably occurred. However, the effects of any consequent residual confounding are likely to have been diminished substantially by the more extensive measurement error related to assessment of exposure to diesel exhaust. Thus, even if there is residual confounding related to measurement error for cigarette smoking, it is likely to be of small magnitude (Kelsey *et al*. 1996).

While asbestos exposure was less common in these study populations, statistical control for this confounder did not eliminate the elevated risk of lung cancer. For example, one study that provides detailed analyses accounting for possible confounding effects of both smoking and asbestos exposure is that of Garshick *et al*. (1987a), which examined a large number of railroad employee deaths (1,256 cases, each matched by sex, age and time of death with two controls). Adjusting for smoking and asbestos exposure resulted in an OR of 1.41 (95% C.I. = 1.06-1.88). In additional analyses using only those workers for whom the investigators had detailed smoking data (n = 758), the ORs for 20 yr of diesel exposure ranged from 1.50-1.53, adjusted for asbestos exposure and several alternative specifications of cigarette smoking history. These models included pack-years as a single continuous variable, as two independent variables (cigarettes per day and years of smoking), or as a categorical variable classified in terms of the number of years the study subject had stopped smoking prior to death. The findings with respect to an association of diesel exposure with lung cancer were robust to model specification, suggesting that confounding by cigarette smoking is unlikely to explain these results. Steenland *et al*. (1990) also found that adjustment for both smoking and asbestos exposure did not eliminate elevated lung cancer risks for long-haul truck drivers.

Diet is another potential exposure that may confound the diesel-lung cancer association among truck drivers. Because long-haul truck drivers tend to be away from home for days at a time, they tend to eat more restaurant meals consisting of fewer fresh fruits and vegetables than the general population, resulting in a lower intake of potentially anti-carcinogenic micronutrients (Wynder and Miller, 1988). While this may be true of long-haul truck drivers, it is not clear that this exposure would be likely to confound all the other diesel-lung cancer associations noted above.

Information bias in these studies predominantly concerns exposure misclassification, which is a common potential problem across all of the studies of cancer and diesel emissions. In nearly all studies, exposure was assigned on the basis of job classification (sometimes just the usual occupation or last job held at retirement). Data on job classification were obtained through company or union records, questionnaires administered to study subjects or their surviving relatives, census or cancer registry data, death certificates or a combination of these sources. Occasionally questions posed to the study subjects or their surrogates focused specifically on diesel exhaust exposure (e.g., Steenland *et al*. 1990), which might result in "recall bias", which occurs when those who have a disease might be more strongly motivated to remember (or even confabulate) potential etiologic exposures (see below). However, most questionnaires asked about the study subjects' job histories, which formed the basis for subsequent assignment of exposure by the study investigators. In these instances, and in those in which job histories were based on existing records, the likelihood of systematic recall bias with regard to occupational diesel exposure is small.

In no case were there direct measurements of diesel exposure of the cohort dating back over the study subjects' working lives. Garshick *et al*. (1987a; 1988) and Woskie *et al*. (1988a; 1988b) attempted to characterize the degree of diesel exposure by job group in their lung cancer studies. However, they were unable to obtain adequate data on historical exposures (Woskie *et al*., 1988b). The presence of misclassified exposures in the studies would tend to bias relative risk estimates towards unity (i.e., towards the null hypothesis of no effect), since exposure misclassification is unlikely to be influenced by whether or not a worker gets cancer. Hence, while exposure misclassification clearly occurs in studies such as these, the result of random misclassification is to underestimate, rather than spuriously elevate, risk estimates. There is no reason to expect any systematic or differential misclassification of railway workers.

Recall bias in next-of-kin interviews could, however, produce systematic misclassification of exposure to diesel exhaust to the extent that more relatives of lung cancer patients than those of controls might have preferentially considered this exposure to be carcinogenic. The overwhelming majority of studies obtaining interview-based data were case-control studies, several of which utilized cancer controls, which would tend to decrease the potential for differential misclassification by disease status of any diesel-related exposure variables (usually occupation title and employment duration). By comparison, the majority of cohort studies relied on tabulated data (e.g. census data, death certificates, job records) to establish occupational history, and would not be susceptible to recall bias. Lung cancer risk estimates available for railroad workers were derived from both cohort and case-control studies. Two of the three

railroad case-control studies utilized cancer controls (Swanson *et al*., 1993; Williams *et al*., 1977) while the third determined diesel exposure by job group (Garshick *et al*., 1987a).

Selection bias may also play a role in helping to explain the findings of the case-control versus the cohort studies. The healthy worker effect is a form of selection bias, which occurs because people who are relatively healthy are more likely than the general population to obtain employment and to stay employed. Although there are various ways to try to account for this bias, they cannot eliminate the original bias introduced by the selection of healthy people into the workforce. In the studies included in this document, the healthy worker effect is manifested, particularly in cohort studies using the general population as the comparison or reference group, by decreased estimates of risk for a variety of outcomes. The healthy worker effect explains in part the lower estimates obtained in the cohort studies relative to the case-control investigations reviewed in this document, and was identified in the meta-analysis as one of the principal sources of heterogeneity in the results of the cohort studies. For example, among the cohort studies with a clear HWE (evidenced by lower than expected all-cause mortality), the pooled diesel-related risk estimates was 1.06, with serious heterogeneity, while for those cohort studies that did not demonstrate a HWE, the pooled risk estimates (under the fixed- and random-effects models) increased to 1.49 and 1.52.

(4) The probability that findings are due to chance. Most studies in Table 6.5 showed an increased risk, though these results were not all statistically significant. Table 6.5 outlines the studies which tested the association between diesel-related occupations and lung cancer, and is divided into studies of truck drivers, transport and heavy equipment workers, dock workers, and railway workers. It can be seen that, with the possible exception of the transport and heavy equipment workers, there is a consistent tendency for point estimates of relative risk to be greater than unity in the studies that accounted for smoking, had an adequate follow-up period, duration of employment, and sufficient statistical power. If these findings were due to chance, one would expect a more nearly equal distribution of point estimates of risk above and below unity.

(5) Evidence for exposure-response relationships. An obvious issue with the majority of occupational studies examining the association between diesel exposure and lung cancer is the lack of quantitative information on diesel exposure. The majority of reported studies rely on job descriptions as a surrogate for exposure status, with only a limited number addressing diesel exhaust specifically. In the studies with sufficient sample size and duration of employment data, several within various occupational categories found significant elevated risks associated with the subgroup having the longest duration of employment, including truck drivers (Hayes *et al*., 1989; Steenland *et al*., 1990; Swanson *et al*., 1993), transportation or heavy equipment operators (Wong *et al*., 1985; Gustavsson *et al*., 1990), dock workers (Emmelin *et al*. 1993), and railroad workers (Garshick *et al*, 1987a; Garshick *et al*., 1988; Swanson *et al*., 1993).

Several studies in which exposure-response relationships were identified involved more thorough attempts by the investigators to examine potential exposures of the study populations, including the two railroad studies by Garshick *et al*. (1987a; 1988) and, to a lesser extent, the study by Howe *et al*. (1983). Two other industry-based studies that developed cumulative diesel-exposure

indices retrospectively also found some evidence of an exposure-response trend, specifically Gustavsson *et al*., (1990) in transportation workers and Emmelin *et al*., (1993) in dock workers.

After adjusting for smoking and asbestos exposure in their lung cancer case-control study, Garshick *et al*. (1987a) found that workers aged less than 64 years (the group with the greatest opportunity for exposure to diesel exhaust) had an OR of 1.64 (95% C.I. = 1.18-2.29) associated with ≥ 20 years of diesel exhaust exposure, and 1.02 (95% C.I. = 0.72-1.45) associated with 5-19 years of exposure. In their retrospective cohort study (which did not control for potential confounding by smoking) Garshick *et al*. (1988) found a RR of 1.72 (95% C.I. = 1.27-2.33) for lung cancer associated with > 15 years of exposure to diesel exhaust, while for 10-14 years the RR was 1.32 (95% C.I. = 1.13-1.56), for 5-9 years it was 1.24 (95% C.I. = 1.06-1.44) and for 1-4 years 1.20 (95% C.I. = 1.01-1.44).

Although no information on smoking status was available, Howe *et al*. (1983) found significantly increased RRs for lung cancer in workers retrospectively classified by their last reported job category as either "probably exposed", RR = 1.35 ($p<0.00l$), or "possibly exposed", RR = 1.20 ($p = 0.013$), as compared to those workers considered "non-exposed" to diesel exhaust. The authors considered these broad classifications to represent relative levels of diesel exposure, which yielded a significant test for trend ($p<0.001$) for increasing risk with increasing likelihood of exposure.

Three of the larger general population studies reported exposure-response relationships -- the prospective cohort study of Boffetta *et al*. (1988) and the case-control studies reported by Swanson *et al*. (1993) and Hayes *et al*. (1989). Boffetta *et al*. (1988) found an exposure-response relationship when diesel-exposed subjects were divided into those exposed for 1-15 years (RR = 1.05, 95% C.I. = 0.80-1.39) versus 16 or more years (RR = 1.21, 95% C.I. = 0.94-1.56) (χ^2 test for trend: $0.05< p <0.10$). This analysis was based on exposure reported in self-administered questionnaires. Hayes *et al*. (1989) reported a significant trend ($p<0.05$) between lung cancer risk (smoking-adjusted ORs) and employment as a truck driver: OR = 1.0 for <9 years, OR = 1.4 for 10-19 years, and OR = 1.5 for ≥ 20 years, with only the longest duration stratum significant (95% C.I. = 1.0-2.3). Swanson *et al*. (1993) reported higher smoking-adjusted odds ratios for lung cancer among white drivers of heavy trucks: OR = 1.4 for 1-9 years, 1.6 for 10-19 years, and 2.5 for ≥ 20 years (test for trend $p<0.05$), again with the longest duration stratum demonstrating statistical significance (95% C.I. = 1.1-4.4). This study found a similar significant trend ($p<0.05$) for employment as a railroad worker: OR = 1.2 for 1-9 years and OR = 2.4 for ≥ 10 years (95% C.I. = 1.1-5.1).

Additionally, as described above and in Appendix C, the meta-analysis identified evidence of exposure-response relationships in the subgroup analyses based on duration of employment. While the initial analysis conducted on all the included studies resulted in elevated pooled risk estimates for strata in which exposure durations were greater than 10 years relative to those with less than 10 years of exposure or for which the exposure durations were not clear from the published reports, there was still significant evidence of heterogeneity for several of the duration strata. In contrast, the analysis utilizing only estimates from the smoking-adjusted studies

showed some evidence of an exposure-response gradient without evidence of statistical heterogeneity. The only occupational subcategory that had sufficient numbers to examine the possibility of an exposure-response trend in the pooled risk estimates was that of truck drivers, for whom the pooled RR estimates were 2.41 (95% C.I. = 1.53-3.81) for exposure durations $\geq$ 20 years (two studies), 1.51 (95% C.I. = 1.18-1.95) for exposure durations <20 years (two studies), and 1.41 (95% C.I. = 1.27-1.58) for those studies in which the exposure duration was unknown (three studies).

(6) Temporality of the associations. That a putative cause precede its effect(s) is a sine qua non for causal inference (Rothman, 1986). It is in this sense that this informal guideline for causal inference is typically used in epidemiology, and is clearly met in the ensemble of diesel exposure studies. One can take consideration of appropriate temporal sequence one step further, and address the issue of latency in the identification of potential cancer-causing agents. Most recognized human carcinogens have a "latent" period of at least l0 years after the initial exposure before their effects can be detected clinically, although increased risks of certain cancers can be identified within five years of exposure (e.g., asbestos and lung cancer). However, the latency for most human carcinogens appears to be 20 years or more. A limitation of the epidemiological studies in humans is the lack of studies with many workers with well characterized exposures of more than 25 to 30 years. Therefore, it is appropriate to consider latency when assessing the likelihood of a causal link between exposure to a given chemical agent or mixture and a neoplastic outcome. Most published studies of occupational diesel exhaust exposure have not meticulously documented potential latency periods. For example, in several studies there was a clearly inadequate allowance for latency between the onset of the study subjects' exposure and the termination of follow-up (Decoufle *et al*. 1977, Kaplan, 1959; Milne *et al*. 1983). These studies were omitted from the meta-analysis described in Appendix C. In many others, the extent of latency was not clearly delineated, but this category of studies contained mixed results, with some showing an increased risk of lung cancer and some that did not(Hayes *et al*. 1989, Damber and Larsson 1987, Pfluger and Minder 1994). Finally, there were several studies that clearly allowed for an adequate latency period, again with a mixture of positive and null results (Garshick *et al*. 1988, Gustafsson *et al*. 1986, Steenland *et al*. 1990).

(7) Biological plausibility of the association. Biological plausibility is not necessary for causal inference from epidemiological studies, since it depends on the state of knowledge of ancillary disciplines. When present, however, supporting evidence from other scientific fields such as toxicology can strengthen the case for a causal association between an exposure and a disease outcome. The basic hypothesis of this review -- that occupational exposure to diesel exhaust causes human lung cancer -- is highly plausible biologically. The evidence can be briefly summarized as follows: (1) Diesel exhaust has been shown to induce lung and other cancers in laboratory animal studies (Brightwell *et al*. 1989; Heinrich *et al*. 1986a; Iwai *et al*. 1986; Mauderly *et al*. 1987a); (2) Diesel exhaust has been shown to contain highly mutagenic substances, including polycyclic aromatic hydrocarbons and nitroaromatic compounds (Ball *et al*. 1990; Gallagher *et al*. 1993; Nielsen *et al*. 1996; Sera *et al*. 1994); (3) Diesel exhaust contains many substances which occur in recognized complex mixtures of human respiratory carcinogens, including cigarette smoke and coke oven emissions (IARC, 1989); and (4) Diesel exhaust contains known and probable human carcinogens.

6.2.4.1 CONCLUSIONS CONCERNING CAUSAL INFERENCE

The epidemiological studies concerning lung cancer risk and exposure to diesel exhaust provide evidence consistent with a causal relationship. The many associations found between lung cancer and diesel exposure are unlikely to be due to chance. Also, with the possible exception of the studies that did not take smoking into account, the findings reviewed above are unlikely to be due to confounding or bias. The results of various studies are consistent in the direction of an effect and are even somewhat similar in magnitude of effect. Although the strength of the associations is weak, reporting relative risk estimates between 1 and 2, several studies show clear exposure-response relationships. As indicated above, this range of relative risk can serve as a basis for causal inference as long as other criteria are met. The temporal relationship between exposures and lung cancer is consistent with a causal relationship. Finally, it is biologically plausible that exposure to diesel exhaust would increase the risk of lung cancer. Therefore, a reasonable and very likely explanation for the increased risks of lung cancer observed in the epidemiological studies is a causal association between diesel exhaust exposure and lung cancer.

Table 6.1.a. Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Syrian Hamsters

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female	Exposed 7-8 hr/d, 5 d/wk for 2 yrs. Diesel exhaust (DE) generated by a 2.4L Daimler-Benz engine using European reference diesel fuel (MMD 0.1 µm). Exposure Groups Group 1: clean air Group 2: 4 mg/m^3 DE Group 3: filtered DE (N=48/group)	Not reported. Median lifetime, (50% of animals surviving), was 72 weeks for all treatment groups.	Tumor incidence not reported for all groups. Groups 1-3: no lung tumors.	Heinrich *et al.* (1982) In: Toxicological Effects of Emissions from Diesel Engines. Ed.: J Lewtas, pp 225-242.
male & female	Exposed for 19 hr/d, 5 d/wk for 120 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine using European reference fuel. Average DE concentration 4 mg/m^3 (MMD 0.35 µm) for unfiltered exhaust flow. Group 1 - control Group 2 - filtered DE Group 3 - total DE (BaP -3 ng/mg particulate) (N=96 per group)	Group 1 males 25% females 2% Group 2 males 33% females 0% Group 3 males 27% females 8%	Group 1 - 0 respiratory tumors Group 2 - 0 respiratory tumors Group 3 - 0 respiratory tumors; increased incidence of bronchiole-alveolar hyperplasia as well as emphysematous lesions	Heinrich *et al.* (1986), J Appl Toxicol, 6(6),383-395. and Stober (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp 421-439.
golden	Exposed for 16 hr/d, 5 d/wk for 2 yrs. DE generated by VW Rabbit 1.5L engine using European standard fuel. Exposure Groups: Group 1: DE 0.7 mg/m^3 Group 2: DE 2.2 mg/m^3 Group 3: DE 6.6 mg/m^3 Group 4: filtered DE (same as 2.2 mg/m^3 group Group 5: filtered DE (same as 6.6 mg/m^3 group) Group 6: control - clean air	Not Reported. Authors stated that due to tan infection significant mortality (45%) occurred between 10 and 12 months. Antibiotics were used to treat the disease in the survivors. Animals were killed at 6, 16, or 24 months	Authors did not report time point specific incidence rates. Total respiratory tumor incidence: Group 1 and 2: not reported Group 3: 1/207 (0.5%) nasal passage tumors Group 4: not reported Group 5: 1/202 (0.5%) trachea tumor, 1/204 (0.5%) lung tumor Group 6: 1/394 (0.25%) larynx tumor, 1/410 (0.24%) lung tumor	Brightwell *et al.* (1989) J Appl Toxicol, 9(1): 23-31.

Table 6.1.b. Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Jackson A	Exposed 20 hr/d, 7 d/wk from 6 wks through 14 wks of age to either clean air or 6 mg/m^3 of DE particulate. Animals were then held in clean air until 9 months of age.	Animals killed at 9 months of age. Survival at end of study: Clean air: male - 18/20 female - 18/20 DE: male - 16/20 female - 18/20	Air: male - 5/18 mice with tumors, 0.33 tumors/mouse female - 11/18 mice with tumors, 0.66 tumors/mouse DE: male - 5/16 mice with tumors, 0.31 tumors/mouse female - 6/18 mice with tumors, 0.50 tumors/mouse	Pepelko and Peirano, (1983) J Am Coll Toxicol, 2(4):253-306.
male, Jackson A	Exposed 8 h/d, 7d/wk from 6 wks until 12 months of age to 12 mg/m^3 raw DE or clean air	initial number of animals unknown. Number of survivors: Air - 38 DE - 44	(initial number of animals unknown) Air: 22/38 mice with tumors, 0.68 tumors/mouse DE: 11/44 mice with tumors, ($p<0.1$), 0.25 tumors/mouse ($p<0.001$)	
male, female Sencar	Parent generation was continuously exposed to clean air or DE from weaning to sexual maturity, and then mated. Exposure was maintained at 6 mg/m^3 from start to exposures through mating, gestation, birth, and weaning. Exposure was increased to 12 mg/m^3 when offspring were 12 wks old and continued until end of study (age 15 months). Survivors were killed at that time. The offspring were subdivided into experimental groups. DE source was 6 cylinder Nissan engine run on Federal Short Cycle. Particles generally 0.1 µm	Offspring were killed at 15 months of age. Survival at end of study: Clean air: male - 105/130 female - 111/130 DE: male - 101/130 female - 104/130	Actual number of animals examined for lung tumors was not specified. Authors reported the following tumor incidences: Air: male - 3.8% lung tumors (3.8% adenomas) female - 7.2% lung tumors (6.3% adenomas, 0.9% carcinomas) DE: males - 5.9% lung tumors (4% adenomas, 2% carcinomas) females - 16.3% lung tumors ($p<0.05$) (16.3% adenomas ($p>0.02$))	Pepelko and Peirano, (1983) J Am Coll Toxicol, 2(4): 253-306.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
Strong A	Strain A pulmonary adenoma assay. Source of DE: 6 cylinder Nissan engine run on Federal Short cycle. Particles generally < 0.1 µm.			Pepelko and Peirano, (1983) J Am Coll Toxicol, 2(4): 253-306.
	Study 1: exposed 20 h/d, 7 d/wk from 6 wks through 14 wks of age to 6 mg/m^3 raw or irradiated DE or air (25/group). Animals were then held in clean air until 9 months of age and killed (male only)	Study 1: Air - 22/25 Raw DE - 19/25 Irradiated DE -22/25	Study 1: Air - 3/22 mice with tumors, 0.13 tumors/mouse. Raw DE - 7/19 mice with tumors, 0.63 tumors/mouse. Irradiated DE - 6/22 mice with tumors, 0.27 tumors/mouse.	
	Study 2: exposed 8 h/d,7 d/wk from 6 wks through 36 or 44 wks of age to 6 mg/m^3 (male only)	Study 2: Air - 403/429 DE - 368/430	Study 2: Air: 73/403 mice with tumors, 0.23 tumors/mouse. DE: 66/368 mice with tumors 0.20 tumors/mice	
	Study 3: exposed 8 h/d, 7 d/wk from 6 wks to 9 months of age to 12 mg/m^3 raw DE or clean air. Exposure took place during the dark cycle. (male and female)	Study 3: Air: male - 97/108 female - 140/142 DE: male - 111/115 female - 139/143	Study 3 Air: Males 28/97 mice with tumors, 0.32 tumors/mouse Females - 31/140 mice with tumors, 0.23 tumors/mouse DE: Males 13/111 mice with tumors, 0.14 tumors/mouse Females - 9/139 mice with tumors, 0.07 tumors/mouse (significant relative to controls, p<0.001)	

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female NMRI	Average DE particulate matter of 4 mg/m^3 for unfiltered exhaust flow. Exposed 19 h/d, 5 d/wk for 120 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine operating on the US 72 test cycle using European reference fuel (MMAD 0.1 µm)		No effects observed in the upper respiratory tract.	Heinrich *et al*. (1986) J Appl Toxicol 6(6): 383-395; Stober (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan and Stober, pp. 421-439.
			lung tumor incidence:	
	Group 1 - control (N=84)	Group 1: 22%	Group 1 11/84 (13%) (9 benign, 2 malignant)	
	Group 2 - filtered DE (N=93)	Group 2: 37%	Group 2 29/93 (31.2%) ($p<0.05$) (11 benign, 18 malignant ($p<0.05$ compared to control))	
	Group 3 - total DE (3 ng BaP/mg particulate (N=76)	Group 3: 5%	Group 3 24/76 (31.6%) ($p<0.05$) (11 benign, 13 malignant ($p<0.05$ compared to control)), increased mortality ($p<0.05$) [time of first tumor not reported]	

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
newborn male, female C57BL/6N	Animals were exposed for 4 hr/d, 4 d/wk to 2-4 mg/m^3 DE (BaP - 0.85 ng/mg; 1-NP - 93 ng/mg) from birth to 28 months. DE generated by YANMAR NSA-40CE, 269 cc engine (MMAD 0.32 µm)	NR (Animals were autopsied at 3, 6, 12, 18, and 28 months)	Lung tumor incidence: 3 - 6 months: Control - 0/6 males and 0.6 females DE - 0/25 males and 0/13 females. 7 - 12 months: Control - 0/4 males and 0/4 females DE - 0/28 males and 0/10 females 13 - 18 months: Control - 0/7 males and 0/12 females DE - 3/40 (7.5%) males (2 adenomas, 1 adenocarcinoma) and 3/39 (7.75) females (2 adenomas, 1 adenocarcinoma). 19 - 28 months: Control - 1/17 males (1 adenoma) and 0/15 females DE - 6/33 (18.2%) males (4 adenomas, 2 adenocarcinomas) and 5/38 (13.2%) females (4 adenomas, 1 adenocarcinoma) 13 - 28 months (males and females): Control - 1/51 (0.02%) DE - 17/150 (11%)	Takemoto *et al* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 311-327.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
newborn male, female ICR	Exposure began within 24 hrs of birth and continued for 28 months. Animals were exposed for 4 hr/d, 4 d/wk to 2-4 mg/m^3 DE (BaP - 0.85 ng/mg; 1-NP - 93 ng/mg. DE generated by YANMAR NSA-40CE, 269cc engine (MMAD 0.32 µm)	NR (Animals were autopsied at 3, 6, 12, 18 and 28 months)	Lung tumor incidence: 3 - 6 month: control - 0/24 males and 0/21 females DE - 0/47 males and 0/22 females. 7 - 12 month: Control - 0/19 males and 0.17 females DE - 0/29 males and 0/20 females. 13 - 18 month: Control - 2/19 (10.5%) males (2 adenomas) and 1/19 (5.3%) females (1 adenoma) DE - 3/15 (20%) males (2 adenoma, 1 adenocarcinoma) 19 - 28 month: Control - 1/10 (10%) males (1 adenoma) and 3/12 (25%) females (2 adenomas, 1 adenocarcinoma) DE - 5/11 (45.5%) males (3 adenomas, 2 adenocarcinomas) and 4/11 (36.4%) females (3 adenomas, 1 adenocarcinoma) 13 - 28 months (males and females): Control - 7/60 (12%) DE - 14/56 (25%); $p < 0.05$ relative to controls	Takemoto et al (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 311-327.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female NMRI	Exposure began at 7 weeks of age, and was performed 18 hr/d, 5 d/wk. All exposures were for 13.5 months, followed by clean air for 9.5 months. A clean air control group was included. Exposure groups: 1) 7 mg/m^3 diesel exhaust 2) carbon black (CB)(7.4 mg/m^3 for 4 months, followed by 12.2 mg/m^3 for 9.5 months) 3) titanium dioxide (TiO_2) (7.2 mg/m^3 for 4 months, followed by 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 5.5 months)	End of treatment mortality: controls: 10% diesel exhaust: 16% carbon black: 20% TiO_2: 30% 50% mortality rate timepoints (months): controls: 20 diesel exhaust: 19 carbon black: 20 TiO_2: 17	Lung adenoma/adenocarcinoma percent incidence controls: 25%/15.4% diesel exhaust: 21.8%/15.4% carbon black: 11.3%/10% TiO_2: 11.3%/2.5% Lung adenomas and adenocarcinomas combined controls: 30% diesel exhaust: 32.1% carbon black: 20% TiO_2: 13.8%	Heinrich *et al*. (1995), Inhal Tox, 7: 533-556.
female NMRI	Exposure began at 7 weeks of age, and was performed 18 hr/d, 5 d/wk, for 23 months. A clean air control group was included. Exposure groups: 1) 4.5 mg/m^3 diesel exhaust 2) The equivalent concentration of diesel exhaust with the particulate matter removed by filtration (particle-free diesel exhaust)	No numerical estimate given for end of treatment mortality. 50% mortality rate timepoints (months): controls: 19 particle-free diesel exhaust: 19 diesel exhaust: 20	Lung adenoma/adenocarcinoma percent incidence controls: 25%/8.8% particle-free diesel exhaust: 31.7%/15% diesel exhaust: 18.3%/5% Lung adenomas and adenocarcinomas combined controls: 30% particle-free diesel exhaust: 46.7% diesel exhaust: 23%	Heinrich *et al*. (1995), Inhal Tox, 7: 533-556.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female C57BL/6N	Exposure began at 7 weeks of age, and was performed 18 hr/d, 5 d/wk, for 24 months, followed by 6 months of clean air. A clean air control group was included. Exposure groups: 1) 4.5 mg/m^3 diesel exhaust 2) The equivalent concentration of diesel exhaust with the particulate matter removed by filtration (particle-free diesel exhaust)	End of treatment mortality: controls: 55% particle-free diesel exhaust: 58% diesel exhaust: 67% 50% mortality rate timepoints (months): controls: 27 particle-free diesel exhaust: 27 diesel exhaust: 25	Lung adenoma/adenocarcinoma percent incidence not stated. Lung adenomas and adenocarcinomas combined controls: 5.1% particle-free diesel exhaust: 3.5% diesel exhaust: 8.5%	Heinrich *et al*. (1995), Inhal Tox, 7: 533-556.
male, female CD-1	Exposure began at 17 weeks of age, and was performed 7 hours/day, 5 days/week for 24 months. A clean air control group was included. Diesel exhaust was generated by 1980 model 5.7-liter Oldsmobile V-8 engines operated continuously on the U.S. Federal Test Procedure urban certification cycle Exposure groups: 1) 0.35 mg/m^3 diesel exhaust 2) 3.5 mg/m^3 diesel exhaust 3) 7.1 mg/m^3 diesel exhaust	50% mortality rate timepoints (days) Males controls 550 group 1 490 group 2 450 group 3 561 Females controls 620 group 1 650 group 2 600 group 3 630	Lung adenomas and adenocarcinomas combined Males controls 7/69 (10.1%) group 1 4/68 (5.9) group 2 5/59 (8.5%) group 3 5/82 (6.1%) Females controls 14/88 (15.9%) group 1 21/103 (23.3) group 2 10/96 (10.4%) group 3 9/104 (8.7%)	Mauderly *et al*. (1996), Fund Appl Tox, 30: 233-242.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice (continued)

Table 6.1.b. Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Mice - Data Summary.

Strain (Sex)	Animals/group	Exposure Duration (hr/d x d/wk x mo)	Particle Concentration (mg/m^3)	Total Lung Tumors (%)	Reference
SENCAR (M)	105	8 x 7[a]	0 (clean air)	3.8	Pepelko and Peirano, (1983)
	101		6.0 - 12.0[b]	5.9	
SENCAR (F)	111	8 x 7[a]	0 (clean air)	7.2	Pepelko and Peirano, (1983)
	104		6.0 - 12.0[b]	16.3	
NMRI (F)	84	19 x 5 x 26	0 (clean air)	13	Heinrich *et al*., (1986)
			4.0[c]	31	
	76		4.0	32	
C57BL/6N (M + F)	51	4 x 4 x 13-28	0 (clean air)	2	Takemoto *et al.*, (1986)
	150	4 x 4 x 13-28	2.0 - 4.0	11	
ICR (M + F)	60	4 x 4 x 13-28	0 (clean air)	12	Takemoto *et al.*, (1986)
	56	4 x 4 x 13-28	2.0 - 4.0	25	
C57BL/6N (F)	120	18 x 5 x 24[d]	0 (clean air)	5.1	Heinrich *et al*., (1995)
	120	18 x 5 x 24[d]	4.5[c]	3.5	
	120	18 x 5 x 24[d]	4.5	8.5	
NMRI (F)	80	18 x 5 x 13.5[e]	0 (clean air)	30	Heinrich *et al*., (1995)
	80	18 x 5 x 13.5[e]	7.0	32.1	
CD-1 (M)	69	7 x 5 x 24	0 (clean air)	10.1	Mauderly *et al*., (1996)
	68	7 x 5 x 24	0.35	5.9	
	59	7 x 5 x 24	3.5	8.5	
	82	7 x 5 x 24	7.1	6.1	
CD-1 (F)	88	7 x 5 x 24	0 (clean air)	15.9	Mauderly *et al*., (1996)
	103	7 x 5 x 24	0.35	5.9	
	96	7 x 5 x 24	3.5	10.4	
	104	7 x 5 x 24	7.1	8.7	

[a] Exposure began at the weaning of the F_1 generation and continued through mating, pregnancy and parturition. F_2 generation exposure continued from weaning to 15 months of age.

[b] Initial diesel particle concentration was 6 mg/m^3 and was increased to 12 mg/m^3 when the offspring were 12 weeks old.

[c] Filtered diesel exhaust at the same volume as the unfiltered diesel exhaust.

[d] Exposure was followed by clean air for up to 6 months. [e] Exposure was followed by clean air for up to 9.5 months.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male Wistar (SPF)	Exposure began at 18 wks of age. 6 hr/d, 5 d/wk for 20 months at 0 or 8.3 mg/m^3 particulate concentration. DE generated by 3 cylinder engine using 2-D/DF-2 fuel (MMAD 0.71 µm). Animals were killed at 4, 8, 16, or 20 months (6 animals/group/time point)	Actual date not reported. Authors stated survival rates not significantly different between groups.	No malignancies reported. Only reported results at 20 month time point (N = 6/group). Control: 1 fibrosarcoma of the subscutis and 1 renal lymphoma. 8.3 mg/m^3 diesel exhaust: 1 mammary fibroadenoma and 1 bronchiolar-adenoma	Karagianes *et al.* (1981) Am Ind Hyg Assoc J 42:382-391.
male Fischer 344	0, 0.25, 0.75, and 1.5 mg/m^3 particulate concentration DE for 20 h/d, 5.5 d/wk, for 9 months, 15 months, or 15 months followed by 8 months of clean air. 30 animals/group. DE generated by GM Oldsmobile 5.7L engines using Type 2D fuel (MMAD 0.2 µm)	Survival not reported.	No lung tumors were observed in control or treatment groups which were examined at 9 or 15 months. No tumors were observed in controls maintained an additional 8 months. Bronchoalveolar carcinomas were reported in treatment groups observed for an additional 8 months: 0.25 mg/m^3 : 1 0.75 mg/m^3 : 3 1.5 mg/m^3 : 1	White *et al.* (1983) J Appl Tox, 3: 332 (letter to editor) and Schreck *et al.* (1981) J Appl Tox, 1:67-76.
male, female Fischer 344	0, 0.7, 2.2 and 6.6 mg/m^3 particulate concentration DE for 16 h/d, 5 d/wk for 2 years, followed by 6 months of clean air. DE generated by two Renault R18 1.6-liter gasoline engines and a VW Rabbit 1.5-liter diesel engine (MMAD 0.14 µm). Interim sacrifices were carried out at 6, 12, 18, and 24 months. Number of animals/sex in control group = 144; number of animals/sex/treatment group = 72.	Survival not reported	Total number of rats with primary lung tumors: control: males 2/134 (1.5%), females 1/126 (0.8%) 0.7 mg/ males 1/72 (1.4%), females 0/71 (0%) 2.2 mg/ males 3/72 (4.2%), females 11/72 (15.3%) 6.6 mg/ males 16/71 (22.5%), females 39/72 (54.2%)	Brightwell *et al.* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober. pp.471-485 and Brightwell *et al.* (1989) J Appl Tox, 9:23-31.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Fischer 344	DE produced by 7 L Caterpillar Model 3304 engine. Exposed for 7 hr/d, 5 d/wk for 24 months to: Group 1: clean air Group 2: 2 mg/m^3 respirable coal dust Group 3: 2 mg/m^3 DE; (BaP content = 13.5 ng/m^3) Group 4: 1 mg/m^3 respirable coal dust + 1 mg/m^3 DE (BaP content = 10.2 ng/m^3)	Not reported (authors stated that no significant differences were seen between groups). All surviving animals killed at 24 months.	Number of animals necropsied/group: 120-121 males and 71-72 females. Specific data not reported. Authors state: "The incidence of neoplasia did not differ statistically (Fischer's exact test) in the four exposures for the fifty tissues examined... No tumors or pre-malignant conditions of the upper airways (mares, larynx, trachea) were found in any rats".	Lewis *et al.*, (1986; 1989)
female Fischer 344/Jcl	Exposed from 5 wks of age for 4 hr/d, 4 d/wk for 24 months. DE generated by YANMAR NSA-40CE 269cc engine (MMAD 0.32 µm). BaP content 0.85 ng/m^3; 1-NP content 93 ng/m^3. Group 1: 2-4 mg/m^3 DE (MMAD 0.32 µm) Group 2: clean air control	NR (Animals were autopsied at 6, 12, 18, and 24 months after the start of inhalation exposure).	No lung tumors were found at the 6 month time point. Lung tumor incidence: <u>12-17 months:</u> group 1 - 0/7 Group 2 - 0/5 <u>18-24 months:</u> Group 1 - 0/15 Group 2 - 0/12	Takemoto *et al.* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober. pp. 311-327.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
SPF Wistar, female	Exposed 19 hr/d, 5 d/wk for 140 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine using European reference fuel. Average DE concentration 4 mg/m^3 for unfiltered exhaust flow. Group 1 - control Group 2 - filtered DE Group 3 - unfiltered DE (BaP content = 13 ng/m^3) (Group 1-3: N=96/group) Group 4 - coal oven flue gas mixed with pyrolyzed pitch 4-7 mg/m^3 (BaP content = 50-100 µg/m^3) (N=48)	Group 1: 45% Group 2: 42% Group 3: 40% Group 4: NR	Group 1: 0/96 lung tumors Group 2: 0/92 lung tumors Group 3: 15/95 (16%) lung tumors Tumors included bronchio-alveolar adenomas, benign and malignant squamous cell tumors. Decreased body weight was noted. Group 4: 21/116 (18.1%) Tumor types were as noted for Group 3.	Heinrich *et al.* (1986) J Appl Tox, 6(6):383-395; Stober (1986) and Mohr *et al.* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan and Stober. pp. 421-439 and 459-470.
male, female Fischer 344/Jcl (SPF)	DE produced by light duty (1.8L unspecified engine) and heavy duty (11L unspecified engine) diesel engines using JIS No. 1 or No. 2 fuel. Animals were exposed for 16 hr/d, 6 d/wk from 5 wks of age. Exposed for 30 months. Light duty (LD) engine exposure groups: 0, 0.1, 0.4, 1, and 2 mg/m^3 (BaP content = 4.4 µg/g diesel exhaust particulate matter ; 1-NP content = 57.1 µg/g) Heavy duty (HD) engine exposure groups: 0, 0.4, 1, 2, and 4 mg/m^3 (BaP content = 2.8 ng/mg; 1-NP content = 15.3 ng/mg)	0 mg/m^3: 192/246 (78%) Light Duty: 0.1 mg/m^3: 109/123 (88.6%) 0.4 mg/m^3: 94/125 (75.2%) 1 mg/m^3: 101/123 (82%) 2 mg/m^3: 94/124 (75.8%) Heavy Duty: 0.4 mg/m^3: 97/123 (78.9%) 1 mg/m^3: 102/125 (81.6%) 2 mg/m^3: 101/123 (82%) 4 mg/m^3: 93/124 (75%)	Animals were killed at 6 month intervals, but the authors did not report time specific incidence rates. Overall lung tumor incidence rates reported were: Light Duty: 0 mg/m^3: 4/123 (3.3%) (1 adenoma, 3 carcinomas) 0.1 mg/m^3: 3/123 (2.4%) (1 adenoma, 2 carcinomas) 0.4 mg/m^3: 1/125 (0.8%) (1 adenoma) 1 mg/m^3: 5/123 (4%) (5 carcinomas) 2 mg/m^3: 3/124 (2.4%) (1 adenoma, 2 carcinomas) Heavy Duty: 0 mg/m^3: 1/123 (0.8%) (1 carcinoma) 0.4 mg/m^3: 1/123 (0.8%) (1 carcinoma) 1 mg/m^3: 0/125 2 mg/m^3: 4/123 (3.3%)(4 carcinomas) 4 mg/m^3: 8/124 (6.5%)(8 carcinomas) ($p<0.05$, significantly greater than control)	Ishinishi *et al* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan and Stober. pp. 329-348.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Fischer 344 (SPF)	Exposed for 8 hr/d, 7 d/wk for 24 months starting at 7 wks of age. Some animals were followed for an additional 6 months. DE generated by 2.4L truck engine. Fuel used not specified. Three exposure groups: Group 1: control Group 2: filtered DE Group 3: 4.9 mg/m^3 DE	2-year survival Group 1: 22/24 (91.7%) Group 2: 16/24 (66.7%) Group 3: 19/24 (70.2%)	Lung Tumor Incidence: < 24 months: Group 1: 0/2; Group 2; 0/8; Group 3: 0/5 24 months Group 1:0/15; Group 2: 0/13; Group 3: 4/14 (28.6%) (2 benign, 2 malignant) > 24 - 30 months: Group 1: 1/22 (benign); Group 2: 0/16; Group 3: 8/19 (5 malignant, 3 benign) Total malignant tumor incidence significantly different in Group 3 relative to controls ($p<0.05$). Malignant Lymphoma Incidence: < 24 months: Group 1: 0/2; Group 2: 5/8 (62.5%); and Group 3: 2/5 (40%). 24 months: Group 1: 0/15; Group 2; 3/13 (23%); and Group 3: 1/14 (7%). > 24 - 30 months: Group 1: 2/22 (9%); Group 2: 4/16 (18.8%); and Group 3: 4/19 (21%). Total malignant lymphoma incidence significantly different in Group 2 and 3 ($p<0.05$) relative to control. Incidence of Tumors in Other Organs: < 24 months: Group 1: 0/2; Group 2: 2/8 (255) (2 benign); Group 3: 1/5 (20%) (1 malignant). 24 months: Group 1: 0/15; Group 2: 4/13 (30.8%) (4 benign); Group 3: 3/14 (21.4%) (3 benign). < 24 - 30 months: Group 1: 2/7 (28.6%) (2 benign); Group 2: 0/3; Group 3: 3/5 (60%) (2 benign, 1 malignant).	Iwai *et al*. (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Ed: Ishinishi, Koizumi, McClellan, and Stober. pp. 349-360

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Fischer 344/Crl (SPF)	DE was generated by 1980 model 5.7L Oldsmobile V-8 engine using D-2 fuel. Animals were exposed to 0, 0.35, 3.5 or 7.0 mg/m^3 DE (MMAD 0.25 µm) for 7 hr/d, 5 d/wk for up to 30 months (surviving animals terminated at 30 months). Exposures started at 17 wks of age.	0 mg/m^3 : approx. 70% 0.35 mg/m^3: NR 3.5 mg/m^3: NR 7.0 mg/m^3: approx. 61% Low and medium group survival rates were intermediate between 0 and 7.0 mg/m^3 rates.	Authors reported overall incidence rates in all animals examined histologically (i.e. includes animals serially sacrificed). Number of animals with tumors/number of animals examined: 0 mg/m^3: all lung tumors - 2/230 (0.8%) (2 adenocarcinoma) 0.35 mg/m^3: all lung tumors - 3/223 (1.3%) (3 adenocarcinomas) 3.5 mg/m^3: all lung tumors - 8/221 (3.6%) (5 adenomas, 1 adenocarcinoma, 2 squamous cell cysts) 7.0 mg/m^3: all lung tumors - 29/227 (12.8%) (1 adenoma, 11 adenocarcinoma, 2 squamous cell carcinomas, 11 squamous cysts). Four animals had two tumors each; 2 animals had 1 adenocarcinoma and 1 squamous cyst each; and 2 animals had 2 adenocarcinomas each. The first tumor was found in a control animal near the 18 month sacrifice time point. Number of animals sacrificed at each time point not specified by authors. See text for more specific data from this study.	Mauderly *et al.* (1987) Fund Appl Tox 9:208-221. (also preliminary results reported in Mauderly *et al.* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober. pp. 397-409.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Wistar	Exposure Groups: 7 mg/m^3 DE 2.5 mg/m^3 DE 0.8 mg/m^3 DE carbon black (7.4 mg/m^3 for 4 months, followed by 12.2 mg/m^3 for 20 months) titanium dioxide (TiO_2) (7.2 mg/m^3 for 4 months, followed by 14.8 mg/ for 4 months and 9.4 mg/m^3 for 16 months) Animals were 7 weeks old when exposure started. All exposures were 18 h/d, 5d/w for 24 months, followed by clean air for 6 months. Diesel exhaust was generated by two 1.6 L Volkswagen diesel engines. One engine (the primary exhaust source) was operated on the U.S. 72 cycle; when necessary, exhaust gas was supplemented by the second engine, which was operated under constant load conditions (2500 U/minute, 40 N). Particle MMAD: 0.25 (diesel exhaust), 0.64 (carbon black) and 0.80 µm (TiO_2).	24 month mortality rates: controls: 42% 0.8 mg/m^3 DE: 45% 2.5 mg/m^3 DE: 52% 7 mg/ DE: 47% carbon black: 56% TiO_2: 60%. 130 week mortality rates(exposure time followed by clean air): controls: 85% 0.8 mg/m^3 DE: 86% 2.5 mg/m^3 DE: 89% 7 mg/ DE: 82% carbon black: 92% TiO_2: 90%	Lung tumors were not observed in the 7 mg/m^3 diesel exhaust, carbon black and TiO_2 exposure groups at 6 and 12 months of exposure (interim sacrifice of approximately 20 animals/group). 30 month (24 months exposure, then 6 months clean air) total lung tumor incidence (with/without benign squamous cysts): controls: 1/217 (0.5%) 0.8 mg/m^3 DE 0/198 2.5 mg/m^3 DE: 11/200 (5.5%) ($P<0.01$ compared to clean air control); 4/200 (2%) 7 mg/m^3 DE: 22/100 (22%); 9/100 (9%); (both $p<0.001$ compared to clean air control) carbon black: 39/100 (39%); 28/100 (28%) TiO_2: 32/100 (32%); 19/100 (19%)	Heinrich *et al*. (1995). Inhalation Toxicology 7:533-556.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.1.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation in Rats (continued)

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Fischer 344	Animals were exposed to DE or carbon black 16 h/d, 5 d/wk for 23 months. Target particle concentrations of diesel exhaust and carbon black (CB) were 2.5 and 6.5 mg/m^3. Diesel exhaust was generated using two 1988 Model LH6 General Motors 6.2-liter V-8 engines operated on the Federal Test Procedure urban certification cycle. Particle size was bimodal for both diesel exhaust and carbon black; the mass median aerodynamic diameter (MMAD) of the large-size mode was 2.0 µm and 1.95 µm for diesel exhaust and carbon black, respectively. The MMAD for the small-size mode was 0.1 µm for both diesel exhaust and carbon black. Approximately 23% and 67% by mass of the diesel exhaust and carbon black particles, respectively, were in the large-size mode.	23 month mortality rates: Males controls: 86% 2.5 mg/m^3 DE: 86% 6.5 mg/m^3 DE: 94% 2.5 mg/m^3 CB: 96% 6.5 mg/m^3 CB: 99% Females controls: 64% 2.5 mg/m^3 DE: 69% 6.5 mg/m^3 DE: 73% 2.5 mg/m^3 CB: 60% 6.5 mg/m^3 CB: 74%	Total lung tumor incidence rates Males controls: 3/109 (2.8%) 2.5 mg/m^3 DE: 5/105 (4.8%) 6.5 mg/m^3 DE: 9/106 (8.5%) 2.5 mg/m^3 CB: 2/106 (1.9%) 6.5 mg/m^3 CB: 4/106 (3.8%) Females controls: 0/105 (0%) 2.5 mg/m^3 DE: 8/105 (7.6%) 6.5 mg/m^3 DE: 29/106 (27.4%) 2.5 mg/m^3 CB: 8/107 (7.5%) 6.5 mg/m^3 CB: 28/105 (26.7%)	Nikula *et al*. (1995) Fund Appl Tox 25:80-94.

Note: All diesel exhaust (DE) exposures are to unfiltered diesel exhaust unless otherwise specified. Concentrations refer to the level of particles in the diesel exhaust.

Table 6.2.a Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Hamsters.

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Syrian	Animals were exposed 7-8 hr/d, 5 d/wk. DE generated by a 2.4L Daimler-Benz engine using European reference diesel fuel (MMAD 0.1 µm). Exposure Groups: Group 1: DEN 1.5 mg/kg subcutaneous (s.c.) injection (N=72) Group 2: DEN 1.5 mg/kg s.c. injection + 4 mg/m^3 unfiltered DE (N=48) Group 3: DEN 1.5 mg/kg s.c. injection + filtered DE (N=57) Group 4: DEN 4.5 mg/kg s.c. injection (N=48) Group 5: DEN 4.5 mg/kg s.c. injection + 4 mg/m^3 unfiltered DE (N=48) Group 6: DEN 4.5 mg/kg s.c. injection + filtered DE (N=60)	not reported (median lifetime, 50% of animals surviving was 72-74 weeks for all treatment groups)	Actual number of animals examined for lung tumors was not specified. Authors reported the following tumor incidences (Note tumor incidence not reported for all groups): Group 1: 13.4 % (larynx/trachea papillomas) Group 2: 1 animal developed a lung tumor Group 3: not reported Group 4: 44.7% (larynx/trachea papillomas) Group 5: 70.2% Group 6: 66%	Heinrich *et al.*, (1982) In: Toxicological Effects of Emissions from Diesel Engines. Ed.: J Lewtas, pp. 225-242.
male, female Syrian	Exposed 19 hr/d, 5 d/wk for 120 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine using European reference fuel. Average concentration 4 mg/m^3 (MMAD 0.35 µm) for unfiltered exhaust flow. Group 1 - Air + 4.5 mg/kg DEN s.c. Group 2 - filtered DE + 4.5 mg/kg DEN s.c. Group 3 - unfiltered DE + 4.5 mg/kg DEN s.c. Group 4 - Air + 250 µg BaP i.t. 1/wk, 20 wks Group 5 - filtered DE + 250 µg BaP i.t. 1/wk, 20 wks Group 6 - unfiltered DE + 250 µg BaP i.t. 1/wk, 20 wks.(N=96 per group)	Not reported.	Group 1 - 10% respiratory tract tumors. Groups 2 & 3 - no significant change with DE. Group 4 - 2% respiratory tract tumors. Groups 5 & 6 - no significant change with DE. Note - no unexposed control group.	Heinrich *et al.* (1986) J Appl Toxicol, 6(6), 383-395. and Stober (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan and Stober, pp. 421-439.

Table 6.2.a Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Hamsters (continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female golden	Exposure of 16 hr/d, 5 d/wk for 2 yrs. DE generated by VW Rabbit 1.5L engine using European standard fuel. Exposure Groups: Group 1: Unfiltered DE 0.7 mg/m^3 + 4.5 mg/kg DEN (s.c.) Group 2: Unfiltered DE 2.2 mg/m^3 + 4.5 mg/kg DEN (s.c.) Group 3: Unfiltered DE 6.6 mg/m^3 + 4.5 mg/kg DEN (s.c.) Group 4: filtered DE (initial particle concentration of 2.2 mg/m^3) + 4.5 mg/kg DEN (s.c.) Group 5: filtered DE (initial particle concentration of 6.6 mg/m^3) + 4.5 mg/kg DEN (s.c.) Group 6: 4.5 mg/kg DEN (s.c.)	Not reported. Authors stated that due to an infection significant mortality (45%) occurred between 10 and 12 months. Antibiotics were used to treat the disease in the survivors. Animals were killed at 6, 16, or 24 months.	Authors did not report time point specific incidence rates; only overall respiratory tumor incidence was reported: Group 1: Males - 3/50 (6%) nasal passage tumor; 7/51 (13.7%) tracheal tumors; 1/50 (2%) lung tumors. Females - 2/50 (4%) nasal passage tumor; 8/50 (16%) tracheal tumor; 1/51 (2%) lung tumor. Group 2: Males - 1/52 (2%) nasal passage tumor; 1/51 (2%) larynx tumor; 12/52 (23%) tracheal tumor; 3/52 (5.8%) lung tumor. Females - 4/49 (8%) nasal passage tumor; 1/48 (2%) larynx tumor; 14/49 (28.6%) tracheal tumor; 3/50 (6%) lung tumor. Group 3: Males - 9/50 (18%) tracheal tumor; 1/51 (2%) lung tumors. Females - 13/48 (27%) tracheal tumor; 3/50 (6%) lung tumors. Group 4: Males - 4/50 (8%) trachea tumor; 3/52 (5.8%) lung tumor. Females - 1/50 (2%) nasal passage tumor; 1/51 (2%) larynx tumor; 5/51 (9.8%) tracheal tumor; 1/52 (1.9%) lung tumor. Group 5: Males - 6/51 (11.8%) tracheal tumor; 5/52 (9.6%) lung tumor. Females - 2/52 (3.8%) nasal passage tumor; 11/52 (21.1%) tracheal tumor; 4/52 (7.7%) lung tumor. Group 6: Males - 1/102 (1%) larynx tumor; 14/101 (13.9%) tracheal tumor; 4/101 (4%) lung tumor. Female - 1/104 (1%) nasal passage tumor; 18/103 (17.5%) tracheal tumor; 3/101 (3%) lung tumor.	Brightwell *et al.*, (1989) J Appl Toxicol, 9(1), 23-31.

NR - not reported
i.t. intratracheal instillation
BaP - benzo[*a*]pyrene
DBahA - dibenz[*ah*]anthracene
DIPN - di-isopropanol-nitrosamine
s.c. subcutaneous injection
i.p. intraperitoneal injection
BHT - butylated hydroxytoluene
DEN - diethylnitrosamine
DPN - dipentylnitrosamine

Table 6.2.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Mice.

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Strong A	Used Strain A pulmonary adenoma assay. Source of DE - 6 cylinder Nissan engine run on Federal Short Cycle. Particles generally < 0.1 µm.	Animals were killed at end of study - 9 months. Survival at end of study:		Pepelko and Peirano, (1983) J Am Coll Toxicol, 2(4): 253-306.
	Study 1: Exposed 8 hr/d, 7 d/wk from 6 wks until 9 months of age to 6 mg/m^3 raw DE or clean air. Half of each exposure group was injected i.p. with a single dose of 1 mg urethane at start of exposure (female only)	Study 1: Air: 58/60 DE: 56/60 Air + urethane: 52/60 DE + urethane: 59/60	Study 1: Air: 4/58 mice with tumors, 0.09 tumors/mouse DE: 14/56 mice with tumors ($p<0.01$), 0.32 tumors/mouse ($p<0.01$) Air + urethane: 9/52 mice with tumors, 0.25 tumors/mouse DE + urethane: 22/59 mice with tumors ($p<0.02$), 0.39 tumors/mouse ($p<0.01$)	
	Study 2: exposed 8 hr/d, 7 d/wk from 6 wks to 9 months of age to 12 mg/m^3 raw DE or clean air. Half of each group was injected i.p. with 5 mg urethane at start of exposure (male and female)	Study 2: Air: male 44/45 female 43/45 DE: male 37/45 female 43/45 Air + urethane: male 38/45 female 37/45 DE + urethane: male 39/45 female 36/45	Study 2: Air: males - 10/44 mice with tumors,0.23 tumors/mouse females - 11/43 mice with tumors, 0.35 tumors/mouse DE: males - 5/37 mice with tumors, 0.19 tumors/mouse females - 4/43 mice with tumors, 0.09 tumors/mouse ($p<0.05$) Air + urethane: males - 32/39 mice with tumors, 2.37 tumors/mouse females - 34/36 mice with tumors, 3.24 tumors/mouse DE + urethane: males - 26/39 mice with tumors, 1.03 tumors/mouse ($p<0.001$) females - 16/36 mice with tumors ($p<0.001$), 0.86 tumors/mouse ($p<0.0001$)	

Table 6.2.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Mice (continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male, female Sencar	Parent generation was continuously exposed to air or DE from weaning to sexual maturity, and then mated. Exposure was maintained at 6 mg/m^3 from start of exposures through mating, gestation, birth, and weaning. Exposure was increased to 12 mg/m^3 when offspring were 12 wks and continued until end of study. Survivors were killed at that time. The offspring were subdivided into groups. DE source was 6 cylinder Nissan engine run on Federal Short Cycle. Particles generally < 0.01 µm.	Offspring were terminated at 15 months of age. Survival at end of study:	Body weight of diesel exposed animals at > 40 wks of exposure was depressed. Decreased survival in diesel exposed groups.	Pepelko & Peirano, (1983) J Am Coll Toxicol 2(4) 253-306
	Group 1: Air or DE plus injected i.p. with BHT once per wk beginning at 7 wks of age for 1 year. Dosage: 300 mg/kg week 1, 83 mg/kg week 2, 150 mg/kg week 3 till termination.	Group 1: (BHT) Air: male 82/130 female 66/130 DE: male 34/130 female 77/130	Group 1: BHT injected: Air: males - 8.5% lung tumors (8.5% adenomas) females - 18.1% lung tumors (16.7% adenomas, 1.5% carcinomas) DE: males - 11.8% lung tumors (8.8% adenomas, 2.9% carcinomas) females - 6.5% lung tumors (p<0.03 compared to air-exposed control) (3.9% adenomas (p<0.01 compared to air exposed control), (2.6% carcinomas)	
	Group 2: Air or DE plus single i.p. injection of 1 mg urethane at 6 weeks of age.	Group 2: (urethane) Air: male 109/130 female 114/130 DE: male 89/130 female 107/130	Group 2: urethane injected: Air: males - 9.2% lung tumors (9.2% adenomas) females - 8.7% lung tumors (7% adenomas, 1.8% carcinomas) DE: male - 10.1% lung tumors (9% adenomas, 1.1% carcinomas) females - 12.1% lung tumors (8.4% adenomas, 3.7% carcinomas)	

Table 6.2.b Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Mice (continued).

Sex/ Strain	Exposure	Survival	Effect/Observations	Reference
female NMRI	Average DE concentration 4 mg/m^3 for unfiltered exhaust flow. Exposed 19 hr/d, 5 d/wk for 120 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine using European reference fuel (MMAD 0.1 µm). Group 1: Air + 50 µg BaP i.t.,1/wk,20 wks (N=64) Group 2: filtered DE + 50 µg BaP i.t., 1/wk, 20 wks Group 3: unfiltered DE + 50 µg BaP i.t., 1/wk, 20 wks Group 4: Air + 100 µg BaP i.t. 1/wk, 10 wks Group 5: filtered DE + 100 µg BaP i.t. 1/wk, 10 wks Group 6: unfiltered DE + 100 µg BaP i.t. 1/wk, 10 wks Group 7: Air + 50 µg DBahA i.t., 1/wk, 10 wks Group 8: filtered DE + 50 µg DBahA i.t., 1/wk, 10 wks Group 9: unfiltered DE + 50 µg DBahA i.t., 1/wk, 10 wks (N=64/group)	Not reported	No effects observed in the upper respiratory tract. Group 1 - 71% lung tumor incidence Group 2 - not reported Group 3 - 41% lung tumor incidence Group 4,5,and 6 - effects not reported. Groups 7,8 and 9 - no significant effects. [time of first tumor not reported]	Heinrich *et al.* (1986), J Appl Toxicol, 6(6),383-395. and Stober (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan & Stober, pp 421-439.

NR - not reported
i.t. intratracheal instillation
BaP - benzo[*a*]pyrene
DBahA - dibenz[*ah*]anthracene
DIPN - di-isopropanol-nitrosamine
s.c. subcutaneous injection
i.p. intraperitoneal injection
BHT - butylated hydroxytoluene
DEN - diethylnitrosamine
DPN - dipentylnitrosamine

Table 6.2.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Rats.

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female SPF Wistar	Exposed 19 hr/d, 5 d/wk for 140 wks starting at 8-10 wks of age. DE generated by unspecified 1.6L automobile engine using European reference fuel. Average DE concentration 4 mg/m^3 for unfiltered exhaust flow.	Mortality in high dose DPN group was increased compared to control and low dose groups. Actual data not reported.		Heinrich *et al.* (1986), J Appl Toxicol, 6(6),383-395. and Stober (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 421-439 and 459-470.
	Group 1 - Air + 500 mg/kg DPN s.c., 1/wk, 25 wks		Group 1: All lung tumors - 45/48 (93.8%) tumors (8 squamous cell carcinomas, 16.7%); Upper respiratory tract - 23/48 (47.9%) benign, 4/48 (8.3%) malignant.	
	Group 2 - filtered DE + 500 mg/kg DPN s.c., 1/wk, 25 wks		Group 2: All lung tumors - 43/48 (89.6%) tumors (7 squamous cell carcinomas); Upper respiratory tract - 11/48 (22.9%) benign ($p<0.05$ compared to control group 1), 6/48 (12.5%) malignant.	
	Group 3 - unfiltered DE + 500 mg/kg DPN s.c., 1/wk, 25 wks		Group 3: All lung tumors - 43/48 (89.6%) (15 squamous cell carcinomas (31.3%) ($p<0.05$ compared to control group 1)); Upper respiratory tract - 7/48 (14.6%) benign ($p<0.05$ compared to control group 1), 8/48 (16.7%) malignant.	
	Group 4 - Air + 250 mg/kg DPN s.c., 1/wk, 25 wks		Group 4: All lung tumors - 39/46 (84.8%) (2 squamous cell carcinomas); Upper respiratory tract - 12/46 (26.1%) benign tumors, 1/46 (2.2%) malignant.	
	Group 5 - filtered DE + 250 mg/kg DPN s.c., 1/wk, 25 wks		Group 5: All lung tumors - 31/46 (67.4%) (2 squamous cell carcinomas); Upper respiratory tract - 2/45 (4.4%) benign ($p<0.05$ compared to control to group 4).	
	Group 6 - unfiltered DE + 250 mg/kg DPN s.c., 1/wk, 25 wks (N=48/grp)		Group 6: All lung tumors - 39/47 (83%) (22 squamous cell carcinomas ($p<0.05$)); Upper respiratory tract - 4/46 (8.7%) benign ($p<0.05$ compared to control to group 4).	

Table 6.2.c Studies of the Carcinogenicity of Diesel Exhaust by Inhalation with Co-administration of Known Carcinogens in Rats (continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Fischer 344/Jcl	Exposed from 5 wks of age for 4 hr/d, 4 d/wk for 24 months. DE generated by YANMAR NSA-40CE 269cc engine (MMAD 0.32 µm). BaP - 0.85 ng/mg 1-NP - 93 ng/mg. Group 1: 1 g/kg DIPN by i.p. injection, 1/wk, 3 wks Group 2: 2-4 mg/m^3 DE + 1 g/kg DIPN by i.p. injection, 1/wk, 3 wks after 1 month of inhalation exposure	Animals were autopsied at 6, 12, 18, and 24 months after the start of inhalation exposure	No lung tumors were found at the 6 month time point. Lung tumor incidence: 12-17 months: Group 1: 2/8 (25%) (2 adenomas) Group 2: 15/18 (83%) (12 adenomas and 3 carcinomas) 18-24 months: Group 1: 14/21 (66.7%) (10 adenomas and 4 carcinomas) Group 2: 19/18 (106%) (12 adenomas and 7 carcinomas) Authors stated that treatment groups were not statistically different from the control group.	Takemoto *et al.* (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 311-327.

NR - not reported
i.t. intratracheal instillation
BaP - benzo[*a*]pyrene
DBahA - dibenz[*ah*]anthracene
DIPN - di-isopropanol-nitrosamine
s.c. subcutaneous injection
i.p. intraperitoneal injection
BHT - butylated hydroxytoluene
DEN - diethylnitrosamine
DPN - dipentylnitrosamine

Table 6.3.a Studies of Carcinogenicity of Diesel Exhaust Particles or Diesel Exhaust Components Administered Intratracheally in Hamsters

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male golden, LAK;LVG	Administered DE particles (DEP), organic extracts of diesel particles (DEE), cigarette smoke condensate (CSC), Coke oven extracts (COE), Roofing tar extracts (RTE), BaP, or saline solution in combination with ferric oxide (FeO) 1/wk for 15 weeks. Group 1: DEP (alone): 1.25, 2.5, or 5.0 mg/wk Group 2: DEP + FeO: 1.25, 2.5 or 5.0 mg/wk Group 3: DEE + FeO: 1.2, 2.5, or 5.0 mg each/wk Group 4: COE + FeO: 1.25, 2.5, or 5.0 mg each/wk Group 6: RTE + FeO: 1.25, 2.5, or 5.0 mg each/wk Group 7: BaP + FeO: 2.0 mg each/wk Group 8: vehicle Group 9: vehicle + FeO: 5.0 mg/wk Group 10: untreated control	Surviving animals were killed at 100 wks. Authors stated survival was not significantly different across treatment groups.	Did not report incidence of neoplastic lesions for 1.25 mg/wk dose groups. Group 1: DEP (alone) 2.5 mg/wk - Lung: 1/47 lymphoma; Pleura: 2/45 malignant mesothelioma: 5.0 mg/wk - Trachea: 3/43 polyp Group 2: DEP + FeO 2.5 mg/wk - Lung: 1/49 adenoma, 1/45 lymphoma, 1/45 polyp; Pleura: 1/43 malignant mesothelioma 5.0 mg/wk - Lung: 1/39 polyp, 1/45 lymphoma, 1/45 undifferentiated malignant tumor (UMT); Pleura: 1/45 UMT; Trachea: 3/45 polyp Group 3: DEE + FeO 2.5 mg/wk - Lung: 2/85 UMT; Pleura: 2/78 UMT, 1/36 malignant mesothelioma 5.0 mg/wk - Lung: 1/48 adenoma; Pleura: 1/43 malignant mesothelioma Group 4: COE + FeO 2.5 mg/wk - Lung: 1/45 adenoma, 1/45 carcinoma 5.0 mg/wk - Lung 1/43 carcinoma, 1/41 adenocarcinoma; Trachea: 1/37 polyp Group 5: CSC + FeO 2.5 mg/wk - Lung: 1/47 adenoma 5.0 mg/wk - Lung: 1/47 lymphoma; Pleura: 1/47 lymphoma; Larynx: 1/42 lymphoma; Trachea: 1/43 lymphoma Group 6: RTE + FeO 5.0 mg/wk - Lung: 1/44 lymphoma, 1/44 reticulosarcoma; Trachea: 2/67 polyp Group 7: BaP + FeO Lung 3/48 polyp, 5/48 carcinoma, 1/42 UMT, 1/42 adenocarcinoma; Pleura: 1/47 myxoma; Larynx: 4/71 polyp; Trachea: 17/72 polyp, 3/39 carcinoma, 2/72 fibroma, 1/33 neoplastic metastases, 1/33 carcinoma, 1/39 sarcoma Group 8: vehicle (alone): 0 incidence Group 9: vehicle + FeO Lung: 1/21 carcinoma, 1/22 reticulosarcoma, 1/22 UMT lung, 1/20 adenoma, 1/22 lymphoma; Pleura: 1/21 reticulosarcoma, 1/22 hemangioma; Larynx: 6/72 polyp; Trachea: 14/81 polyp, 4/39 carcinoma, 1/39 fibroma, 1/39 sarcoma Group 10: untreated control Lung: 2/96 lymphoma, 1/95 UMT; Pleura: 1/95 lymphoma; Larynx: 1/90 polyp	Shefner *et al*., EPA-600/1-85-004; Jan 1985

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice

(continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
golden	Animals were dosed with 0.5 mg 1,6-dinitropyrene (DNP) or saline 1/wk for 26 weeks. Animals were observed for 48 weeks.	not reported	Tumor Incidence: 1,6-DNP: 19/20 (955) adenocarcinoma of the lung and 12/20 (60%) leukemia Saline: 0/20 and 0/20	Sato *et al*. (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober,pp 271-277
male golden	Administered tar from heavy duty diesel engine exhaust (DET), cigarette smoke condensate (CSC) or control vehicle solution 1/wk for 15 wks. Animals were then observed for remaining lifespan. Group 1: control (N=59) Group 2: 1 mg DET/wk, total 15 mg Group 3: 0.5 mg DET/wk, total 7.5 mg Group 4: 0.1 mg DET/wk, total 1.5 mg Group 5: 0.1 mg DET/wk + 0.5 mg BaP/wk Group 6: 0.5 mg BaP/wk, total 7.5 mg Group 7: 2.03 ng BaP/wk, total 0.03 µg Group 8: 1 mg CSC/wk, total 15 mg (N=62/grp for Groups 2-8)	not reported; (15 wk survival 87-98% in all groups except Group 2 - 71% survival)	Respiratory Tumor Incidence: Group 1: 1/58 (1.7%) (1 benign lung tumor) Group 2: 1/44 (2.3% (1benign larynx tumor) Group 3: 0/56 Group 4: 1/59 (1.7%) (1 benign larynx tumor) Group 5: 52/57 (91.2%) (lung tumors - 42 malignant; trachea tumors - 26 malignant, 8 benign; larynx tumors - 1 benign) Group 6: 45/51 (88.2%) (Lung tumors - 34 malignant, 1 benign; trachea tumors - 17 malignant, 7 benign; larynx tumors - 1 benign) Group 7: 1/58 (1.7%) (1 benign larynx tumor) Group 8: 1/55 (1.8%) (1 benign lung tumor)	Kunitake *et al*. (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 235-252.

Table 6.3.b Studies of Carcinogenicity of Diesel Exhaust Particles or Diesel Exhaust Components Administered Intratracheally in Rats

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Osborne-Mendel	Implanted various DE condensate fraction and BaP in beeswax and trioctanoin into the lungs.	Median survival time, wks:	Number of tumors (SCC = squamous cell carcinomas; BAA = bronchiolar/alveolar adenoma);	Grimmer *et al.*(1987) Can. Let., 37:173-180.
	Group 1 Fraction I: Hydrophilic fraction 6.7 mg	Group 1 Fraction I - 97	Group 1 Fraction I - 0 SCC and 1 BAA	
	Group 2 Fraction II: Hydrophobic fraction 20.0 mg	Group 2 Fraction II - 99	Group 2 Fraction II - 5 SCC (14.2%) and 0 BAA	
	Group 3 Fraction IIa: non-aromatics + polyaromatic compounds (PAC) 2 and 3 rings 19.22 mg	Group 3 Fraction IIa - 103	Group 3 Fraction IIa - 0 SCC and 1 BAA	
	Group 4 Fraction IIb: polyaromatic hydrocabons (PAH) 4 - 7 rings 0.21 mg	Group 4 Fraction IIb - 102	Group 4 Fraction IIb - 6 SCC (17.1%) and 0 BAA	
	Group 5 Fraction IIc: polar PAC 0.29 mg	Group 5 Fraction IIc - 97	Group 5 Fraction IIc - 0 SCC and 0 BAA	
	Group 6 Fraction IId: nitroPAH 0.19 mg	Group 6 Fraction IId - 106	Group 6 Fraction IId - 1 SCC (2.8%) and 0 BAA	
	Group 7 Reconstituted: hydrophobics (Fraction IIa, b, c) 19.91 mg	Group 7 Reconstituted - 93	Group 7 Reconstituted - 7 SCC (205) and 1 BAA	
	Group 8 Control I: untreated	Group 8 Control I - 110	Group 8 Control I - 0 SCC and 0 BAA	
	Group 9 Control II: treated with beeswax and trioctanoin	Group 9 Control II - 103	Group 9 Control II - 0 SCC and 1 BAA	
	Group 10 BaP: 0.03, 0.1, or 0.3 mg	Group 10 BaP: 0.03 mg - 97 0.1 mg - 98 0.3 mg - 69	Group 10 BaP: 0.03 mg - 3 SCC (8.6%) and 0 BAA 0.1 mg - 11 SCC (31.4%) and 0 BAA 0.3 mg - 27 SCC (77.1%) and 0 BAA	

Table 6.3.b Studies of Carcinogenicity of Diesel Exhaust Particles or Diesel Exhaust Components Administered Intratracheally in Rats (continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Fischer 344, SPR		Survival at 30 months:		Kawabata *et al*. (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan, and Stober, pp. 213-22
	Group 1: 1 mg DE particles/wk, 10 wks	Group 1 - 20/42 (47.6%)	Group 1: 31/42 (73.8%) ($p<0.01$) animals with 37 lung tumors (11 benign; 26 malignant, $p<0.01$). Also significantly different from Group 2.	
	Group 2: 1 mg activated carbon/wk, 10 wks	Group 2 - 10/23 (34.8%)	Group 2: 11/23 (47.8%) lung tumors ($p<0.01$) (4 benign; 7 malignant, $p<0.01$)	
	Group 3: 0.2 ml vehicle/wk, 10 wks	Group 3 - 8/23 (34.8%)	Group 3: 1/23 (4.3%) lung tumors (1 malignant	
	Group 4: No treatment	Group 4 - 14/44 (31.8%)	Group 4: 0/44 lung tumors	
	Animals were examined 30 months after instillation.			

Table 6.3.b Studies of Carcinogenicity of Diesel Exhaust Particles or Diesel Exhaust Components Administered Intratracheally in Rats (continued).

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
female Wistar Crl: (WI) BR	One exposure/week; group descriptions below include material description, # treatments × dose, total dose. Control: vehicle (saline + Tween 80), = 3 × 0.2 ml, 13 × 0.3 ml (4.5 ml total). $DS_{original}$: unextracted diesel soot, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg). DS_{30}: extracted diesel soot, 3 × 2.0 mg, 2 × 3.0 mg, 12 × 1.5 mg (total dose 30 mg). DS: extracted diesel soot, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg). Pr: Extracted Printex 90, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg). LB: extracted Lamp Black 101, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg). BaP_{30}: benzo[*a*]pyrene, 3 × 2.0 mg, 2 × 3.0 mg, 12 × 1.5 mg (total dose 30 mg). BaP: benzo[*a*]pyrene, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg). DS + BaP: extracted diesel soot + benzo[*a*]pyrene, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg [including 170 µg BaP]). Pr + BaP: extracted Printex 90 + benzo[*a*]pyrene, 3 × 0.66 mg, 13 × 1.0 mg (total dose 15 mg[including 443 µg BaP]).	Mortality at 500 days: Control, $DS_{original}$, DS_{30}, DS, Pr, LB, BaP, DS + BaP, Pr + BaP: approximately 25 - 33%. BaP_{30}: approximately 80%.	Benign and malignant lung tumor incidence: Control: 0/47 (0%) $Ds_{original}$: 8/48 (17%) DS_{30}: 10/48 (21%) DS: 2/48 (4%) Pr: 4/48 (21%) LB: 4/48 (8%) BaP_{30}: 43/47 (90%) BaP: 12/48 (25%) DS + BaP: 4/48 (8%) Pr + BaP: 13/48 (27%) Cystic keratinizing epitheliomas (squamous cysts) were included as benign tumors in the tumor incidence	Dasenbrock *et al*. (1996). Tox. Lett., 88:15-21.

Table 6.3.C Studies of Carcinogenicity of Diesel Exhaust Particles or Diesel Exhaust Components Administered Intratracheally in Mice

Sex/Strain	Exposure	Survival	Effect/Observations	Reference
male ICR	Titanium dioxide (TiO_2), hexane/benzene/methanol-washed diesel exhaust particles (WDEP) or unwashed diesel exhaust particles (DEP) were suspended in 50 mM phosphate-buffered saline. Mice were injected intratracheally with 0.1 mg of appropriate particle. Control animals were injected with 0.1 ml of vehicle. Injections were performed 10 times at weekly intervals.	Number of animals surviving to 12 months: controls 21 (78%) TiO2 20 (74%) WDEP 24 (89%) DEP 20 (74%)	Number of lung tumors (adenomas and adenocarcinomas) controls 3/27 (11%) TiO_2 5/27 (16%) WDEP 7/27 (26%) DEP 9/26 (35%)	Ichinose *et al*. (1997) Int. J. Oncology, 11: 571-575.

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice

Strain: SENCAR
Survival: not specified
Reference: Nesnow *et al*. (1982) JNCI, 68(5),829-834; Nesnow *et al*. (1982) In: Toxicological Effects of Emissions from Diesel Engines, Ed: J Lewtas, pp. 295-320; and Nesnow *et al*. (1983) Env Health Perspec, 47, 255-268.

Exposure	Effect/Observations
Tumor Initiation Studies: Extracts from 4 diesel engines, 1 gasoline engine, roofing tar emissions, and coke oven emissions were applied as a single dose (except for the highest dose which was given in 5 daily doses) to 7-9 week old mice. One week after treatment all groups were exposed to 2 μg/animal TPA 2/wk. <u>1973 Datsun-Nissan 220C:</u> 0.1, 0.5, 1.0, 2.0, or 10.0 mg/animal (contained 96.2 ng BaP/mg exhaust particle) <u>1978 Oldsmobile 350:</u> 0.1, 1.0, 2.0, or 10.0 mg/animal (contained 0.4 ng BaP/mg) <u>prototype Volkswagen turbo-charged Rabbit:</u> 0.1, 0.5, 1.0, 2.0, or 10.0 mg/animal (contained 4.6 ng BaP/mg)	Authors did not report statistical significance. Scored for papillomas at 6 months and for carcinomas at 1 yr after initiation. % Mice with Papillomas (combined males and females) (number examined); Papillomas/Mouse; % Mice with Carcinomas; and Carcinomas/Mouse: <u>Datsun-Nissan engine:</u> 0.1 mg: 1.5% (76), 0.015/mouse, 2.56%, and 0.026/mouse 0.5 mg: 24.5% (77), 0.365/mouse, 11.6% and 0.116/mouse 1.0 mg: 35.9% (78), 0.453/mouse, 16.6% and 0.166/mouse 2.0 mg: 61.7% (75), 1.37/mouse, 14.1% and 0.141/mouse 10 mg: 93.5% (75), 5.6/mouse, 33.5% and 0.35/mouse. <u>Oldsmobile engine:</u> 0.1 mg: 14% (80), 0.155/mouse, 8% and 0.1/mouse 1.0 mg: 22% (79), 0.304/mouse, 8% and 0.08/mouse 2.0 mg: 30% (80), 0.375/mouse, 4% and 0.04/mouse 10 mg: 15.5% (78), 0.17/mouse, 10.5% and 0.105/mouse. <u>VW Rabbit engine:</u> 0.1 mg: 16.1% (77), 0.161/mouse, 0% 0.5 mg: 9.32% (77), 0.093/mouse, 0% 1.0 mg: 19.7% (76), 0.238/mouse, 3% and 0.03/mouse 2.0 mg: 17.6% (73), 0.206/mouse, 5.5% and 0.055/mouse 10.0 mg: 33% (76), 0.405/mouse, 7.5% and 0.075/mouse.

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Strain: SENCAR
Survival : not specified
Reference: Nesnow *et al*. (1982) JNCI, 68(5),829-834; Nesnow *et al*. (1982) In: Toxicological Effects of Emissions from Diesel Engines, Ed: J Lewtas, pp. 295-320; and Nesnow *et al*. (1983) Env Health Perspec, 47, 255-268.

Exposure	Effect/Observations
Mercedes 300D: 0.1, 0.5, 1.0, 2.0 or 10.0 mg/animal (BaP content not reported) 1972 heavy duty Caterpillar 3304: 0.1, 0.5, 1.0, 2.0, or 10.0 mg/animal (contained 0.5 ng BaP/mg) Residential furnace (Model 125-OU-AC-A): 0.1, 0.5, 1.0, 2.0, or 10.0 mg/animal (BaP content not reported)	Mercedes engine: 0.1 mg: 9.05% (77), 0.091/mouse, 0% 0.5 mg: 10.7% (68), 0.107/mouse, 2.13% and 0.021/mouse 1.0 mg: 22% (79), 0.363/mouse, 2.53% and 0.025/mouse 2.0 mg: 7.9% (78), 0.089/mouse, 0% 10.0 mg: 25.7% (78), 0.321/mouse, 2.44% and 0.024/mouse. Heavy-duty Caterpillar engine: 0.1 mg: 5.06% (77), 0.076/mouse, 2.53% and 0.025/mouse 0.5 mg: 9% (77), 0.105/mouse, 0% 1.0 mg: 9.9% (79), 0.099/mouse, 3% and 0.03/mouse 2.0 mg: 8% (77), 0.08/mouse, 1.48% and 0.015/mouse 10.0 mg: 7.5% (79), 0.075/mouse, 0%. Residential furnace: 0.1 mg: 0%, 0/mouse, 5.5% and 0.055/mouse 0.5 mg: 7.53% (79), 0.073/mouse, 5.53% and 0.055/mouse 1.0 mg: 10.5% (80), 0.115/mouse, 1.5% and 0.015/mouse 2.0 mg: 3.99% (79), 0.04/mouse, 3% and 0.03/mouse 10 mg: 23.05% (78), 0.336/mouse, 6.5% and 0.065/mouse.
BaP: 2.5, 12.6, 50.5, or 100.9 µg/animal TPA: No initiator dose	BaP: 2.52 µg: 38.1% (79), 0.47/mouse, 5% and 0.06/mouse 12.62 µg: 65.3% (77), 1.46/mouse, 21.4% and 0.214/mouse 50.46 µg: 87.3% (79), 4.28/mouse, 22.5% and 0.225/mouse 100.92 µg: 96% (76), 9.05/mouse, 27.5% and 0.29/mouse. TPA (no initiator dose): 6.46% (76), 0.065/mouse, 2.4% and 0.024/mouse.

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Strain: SENCAR
Survival : not specified
Reference: Nesnow *et al*. (1982) JNCI, 68(5),829-834; Nesnow *et al*. (1982) In: Toxicological Effects of Emissions from Diesel Engines, Ed: J Lewtas, pp. 295-320; and Nesnow *et al*. (1983) Env Health Perspec, 47, 255-268.

Exposure	Effect/Observations
Complete Carcinogen Study. Extracts from Nissan, Oldsmobile, and Caterpillar diesel engines, roofing tar emissions, coke oven emissions and BaP were applied 1/wk, except for the highest dose which was given 2/wk, for 50-52 weeks. 1973 Datsun-Nissan 220C: 0.1, 0.5, 1.0, 2.0, or 4.0 mg/animal/week Oldsmobile: 0.1, 0.5, 1.0, 2.0, or 4.0 mg/animal/week Caterpillar diesel engine: 0.1, 0.5, 1.0, 2.0, or 4.0 mg/animal/week Roofing tar: 0.1, 0.5, 1.0, 2.0, or 4.0 mg/animal/week Coke oven: 0.1, 0.5, 1.0, 2.0, or 4.0 mg/animal/week BaP: 12.6, 25.2, 50.5, 101, or 202 µg/animal/week	Scored for carcinomas at 1 yr; % Mice with Carcinomas; and Carcinomas/Mouse: Nissan Diesel engine: 0.1 mg - 2 mg: 0% and 0/mouse 4 mg: 4% and 0.04/mouse. Oldsmobile: all doses, except 0.5 mg, had 0% incidence. 0.5 mg : 1.5% and 0.015/mouse. Caterpillar diesel engine: 0.1 mg: 1.5% and 0.015/mouse 0.5 - 4.0 mg: 0% incidence. Roofing Tar: 0.1 and 0.5 mg: 0% and 0/mouse 1.0 mg: 1.5% and 0.015/mouse 2.0 mg: 5.5% and 0.055/mouse; 26.5% and 0.28/mouse. Coke Oven: 0.1 mg: 5% and 0.05/mouse 0.5 mg: 33% and 0.33/mouse 1.0 mg: 54% and 0.575/mouse 2.0 mg: 80% and 0.89/mouse 4.0 mg: 86.5% and 0.915/mouse. BaP: 12.6 µg: 9% and 0.09/mouse 25.2 µg: 53% and 0.53/mouse 50.5 µg: 95.5% and 0.955/mouse 101 µg: 85% and 0.905/mouse 202 µg: 86.5% and 0.89/mouse Control: 0% and 0/mouse

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Strain: C3H/HeJ
Survival : not reported (Some animals were still alive at time of manuscript publication).
Reference: Depass *et al*. (1982) In: Toxicological Effects of Emissions from Diesel Engines, Ed: J Lewtas, pp. 321-323

Exposure	Effect/Observations
Tumor Initiation Studies: A single initiating dose was followed by repeated application of 0.01% PMA. DE particulate generated by a GM Oldsmobile 350 D engine. Initiator dose groups (N=40/grp): DE particulate: 10% suspension (2.0 mg/day) DE particulate extract: 50% suspension (12.0 mg/day) Acetone: vehicle control PMA: no initiator treatment	<u>Number of tumor bearing animals:</u> Diesel particulate: 3 Diesel particulate extract: 3 Acetone control: 1 PMA control: 2
Tumor Promotion Studies. Single initiating dose of 1.5% BaP was followed by repeated application of one of the following (N=40/grp): DE particulate: 10% suspension (2.0 mg/day) DE particulate extract: 25% suspension (5.1 mg/day) or 50% suspension (12.0 mg/day) Acetone control PMA (positive control): 0.01% suspension ($1.5x10^{-3}$ mg/day) BaP: no additional treatment	<u>Number of tumor bearing animals:</u> Diesel particulate: 0 Diesel particulate extract: 25% suspension - 2; 50% suspension - 1 Acetone control: 0 PMA: 19 ($p<0.001$) Animals also exhibited shorter survival time. BaP: 0

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Strain: C3H/HeJ
Survival : not reported (Some animals were still alive at time of manuscript publication).
Reference: Depass *et al*. (1982) In: Toxicological Effects of Emissions from Diesel Engines, Ed: J Lewtas, pp. 321-323

Exposure	Effect/Observations
Complete Carcinogenesis Studies: Repeated doses of diesel particulate, diesel particulate extract, BaP or acetone were administered. Dose groups: <u>Diesel particulate:</u> 5% (1.0 mg/day) suspension 10% (2.0 mg/day) suspension <u>Diesel particulate extract:</u> 5% (1.0 mg/day) 10% (2.2 mg/day) 25% (5.1 mg/day) 50% (12.0 mg/day) suspension <u>BaP:</u> 0.2% suspension (0.038 mg/day) Acetone: vehicle control	<u>Number of tumor bearing animals:</u> <u>Diesel particulate:</u> 5% - 0 10% - 0 <u>Diesel particulate extract:</u> 5% - 0 10% - 0 25% - 0 50% - 1 <u>BaP:</u> 38 ($p<0.001$) <u>Acetone:</u> 0

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Sex/Strain: female ICR (SPF)
Survival : not reported
Reference: Kunitake *et al*., (1986) In: Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust. Eds: Ishinishi, Koizumi, McClellan and Stober, pp. 235-252.

Exposure	Effect/Observations
Initiators: heavy duty diesel exhaust tar (DET), benzo(a)pyrene (BaP), cigarette smoke condensate (CSC). Promoter: TPA. Initiating doses were given in 10 subdoses, 1 every other day. Followed 1 week later by promoter (2.5 µg TPA, 3/wk, 25 wks). Initiating dose groups (total dose) DET: 45 mg DET (contained 0.002 µg BaP/mg) 15 mg DET 5 mg DET DET + BaP: 45 mg DET + 1.7 µg BaP 15 mg DET + 0.57 µg BaP 5 mg DET + 0.19 µg BaP CSC: 45 mg CSC (contained 0.001 µg BaP/mg CSC) Vehicle Control: Acetone BaP: 1.8 µg	No skin cancer was found in any exposure group. Incidence of skin tumors (papillomas) DET: 45 mg: 4/50 (8%) 15 mg: 1/48 (2.1%) 5 mg: 0/49 DET + BaP: 45 mg + 1.7 µg: 5/50 (10%) 15 mg + 0.57 µg: 2/46 (4.3%) 5 mg + 0.19 µg: 1/49 (2%) CSC: 1/44 (2.3%) Vehicle Control: 0/50 BaP: 0/50

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Strain: SENCAR
Survival : not reported
Reference: Nesnow *et al*., (1984) Cancer Letters 23:1-8.

Exposure	Effect/Observations
Tumor Initiation Studies. Study duration 30 weeks	Mice with papillomas and papillomas/mouse (at 30 weeks)
Experiment 1: various doses of 1-NP were applied topically. One wk after treatment all groups were exposed to 2 µg/animal TPA 2/wk, for 30 wks. 1-NP Dose Groups: 0.03, 0.1, 0.3, 1.0 or 3.0 mg/animal) (all doses, except 3.0 mg, given as single application. BaP: 0.051 mg Control: Acetone only	1-NP: 0.03 mg: 8.6% of 74 animals and 0.086/mouse 0.1 mg: 13% of 80 animals and 0.23/mouse 0.3 mg: 4.05% of 77 animals and 0.041/mouse 1.0 mg: 10.4% of 77 animals and 0.105/mouse 3.0 mg: 13% of 77 animals and 0.154/mouse BaP: 0.051 mg: 100% of 72 animals and 7.4/mouse Control: 2.65% of 70 animals and 0.0265/mouse
Experiment 2: various doses of a mixture of 1,3-DNP, 1,6-DNP, and 1,8-DNP (1:1.94:1.95) were applied topically. One week after treatment all groups were exposed to 2 µg/animal TPA 2/wk, for 30 weeks. Mixture of DNPs: 0.05, 0.1, 0.5, 1.0, or 2.0 mg/animal (doses 0.05 & 0.1 mg given as single dose; doses 0.5 & 1.0 given 1/d for 5 days; dose 2.0 mg given 2/d, 5 days) BaP: 0.051 mg Control: Acetone only	DNPs Mixture: 0.05 mg: 3% of 77 animals and 0.066/mouse 0.1 mg: 1.52% of 75 animals and 0.015/mouse 0.5 mg: 2.75% of 74 animals and 0.0275/mouse 1.0 mg: 15.5% of 77 animals and 0.26/mouse 2.0 mg: 27.5% of 77 animals and 0.38/mouse BaP (0.051 mg): 53.8% of 37 animals and 1.13/mouse Control (Acetone): 0% and 0/mouse

Table 6.4. Summary of Experimental Studies of Diesel Exhaust and Diesel Exhaust Components Carcinogenicity Following Skin Application in Mice (continued).

Sex/Strain: female Crl/CD-1 (ICR)BR
Survival : not reported
Reference: Nesnow *et al*., (1984) Cancer Letters 23:1-8.

Exposure	Effect/Observations
Tumor Initiation Study: Initiating doses were given in 10 subdoses, 1 every other day. Dosing was followed 10 days later by application of promoter, TPA (2.5 µg, 3/wk for 25 weeks). Initiator dose groups (total dose): BaP - 0.05 mg/mouse 6-NBaP - 0.05 mg/mouse Chrysene - 1.0 mg/mouse 6-NC - 1.0 mg/mouse Perylene - 1.0 mg/mouse 3-NPerl - 1.0 mg/mouse Pyrene - 1.0 mg/mouse 6-NP - 1.0 mg/mouse Vehicle Control (N = 20/group)	% Skin tumor bearing animals, skin tumor/mouse: BaP: 90% ($p<0.01$) and 7.1 tumors/mouse 6-NBaP: 25%* and 0.3 tumors/mouse Chrysene: 100% ($p<0.01$) and 7.7 tumor/mouse 6-NC: 60%* ($p<0.01$) and 2.1 tumor/mouse Perylene: 20% and 0.2 tumor/mouse 3-NPerl: 42% * ($p<0.01$) and 0.5 tumor/mouse Pyrene: 20% and 0.2 tumor/mouse 1-NP: 16% and 0.2 tumor/mouse Vehicle Control: 5% and 0.1 tumor/mouse * - also significantly different from parent compound.

TPA - tetradecanoylphorbol acetate
PMA - phorbol myristate acetate
6-NBaP - 6-nitrobenzo[a]pyrene
6-NC - 6-nitrochysene
3-NPerl - 3-nitroperylene
1-NP - 1-nitropyrene

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Menck and Henderson, 1976 USA	Cohort Truck drivers	 109	SMR 1.65	 p < 0.01	Included 2,161 lung cancer cases identified from death certificates in white males, aged 20 to 64, from 1968 through 1970, and 1777 incident cases of lung cancer reported to LA County Cancer Surveillance Program for 1972 - 73. Occupational information obtained from death certificates or hospital admission sheets/medical records represented the last occupation and industry of employment. No data on smoking.
Decoufle *et al*. 1976 USA	Case-control Truck or tractor driver ≥ 5 years as truck, bus or taxi driver	 56 50	OR 1.07 0.89	 N.S. N.S.	Hospital-based study of 6,434 cancers cases admitted to Roswell Park Memorial Institute between 1956 and 1965. Controls were patients admitted with non-neoplastic disease. Occupation and smoking data obtained by questionnaire. Crude adjustment for smoking. Inadequate latency.
Williams *et al*. 1977 USA	Case-control Transportation Industry Truck drivers Railroad workers Truck Industry	 38 22 12 13	RR 1.17 1.52 1.40 1.34	 N.S. N.S. N.S. N.S.	Study examined cancer incidence and its relation to occupation and industry based on the U.S. 3rd National Cancer Survey. The number of cases of cancer at various sites were compared with that of cases at all other sites combined. Occupational history (main and recent employment) and data on smoking were obtained by interview (n=7,518). IARC noted the potential bias in this study due to the relatively low level of response to the questionnaire (57%). Results were controlled for tobacco use, alcohol consumption, race, education and geographic location.
Leupker and Smith 1978, USA	Cohort Total cohort Age 50-59	 34 Not given	SMR 1.21 1.37	 N.S. p<0.001	Death certificates for a 3-month period in 1976 in the Central States Teamster population were examined. Comparison group was the US male population and was not adjusted for race. No data on smoking. Authors noted the follow-up was short. Retirees and members with lapsed benefits were excluded. 48,358 members were eligible in the 50-59 age group.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Ahlberg *et al*. 1981 SWEDEN	Cohort All truck drivers* Stockholm truck drivers[#]	 161	RR 1.33 1.62	 1.13-1.56 1.15-2.28	Cohort consisted of 34,027 Swedish drivers considered to be exposed to diesel exhaust identified from the 1960 national census. Reference population consisted of blue-collar workers from the same census thought to have had no exposure to petroleum products or chemicals (n=686,708). No data on smoking; however, a study of 470 professional drivers in Stockholm found that 78% of fuel truck drivers and 31% of other truck drivers smoked compared to 40% in the Swedish population (citing unpublished study). [#]Subset of all non-fuel tank drivers. *Does not include fuel tank drivers.
Milne *et al*. 1983 USA	Case-control Occupational groups: All transport operatives Bus drivers Truck drivers Other transport Industry groups: Railroad	 36 4 23 7 34	OR 1.3 (1.1)* 3.5 (2.8)* 1.6 (1.3)* 0.7 (0.6)* 0.8 (0.8)*	 N.S. p≤0.05* p≤0.05* N.S. N.S.	Study compared lung cancer deaths with mortality from all other cancers in Alameda County between 1958 and 1962 to investigate possible associations between lung cancer and occupation. Data on cause of death and occupation were obtained from death certificates. No data on smoking or the types of vehicle engines. Results reported are for males. *Results in parentheses are ORs with potential occupationally related cancer removed from the control population. Significant risk estimates only observed when compared with control group before such cancers removed.
Hall and Wynder 1984 USA	Case-control Usual employment: Total diesel- exposed - adjusted for smoking Selected occupations: Truck drivers Railroad workers Heavy equipment repairmen & operators - adjusted for smoking Smoking & DE exposure: Non & ex-smokers ≤ 20 cigarettes/day > 20 cigarettes/day	 45 22 5 10 10 7 21	OR 2.0 1.4 1.4 2.6 3.5 1.9 1.46* 0.82* 1.30*	 1.2-3.2 0.8-2.4 0.7-2.6 0.5-12.8 1.0-11.8 0.6-5.5 0.9-2.3 0.5-1.4 0.8-2.1	Study consisted of 502 men with histologically confirmed primary lung cancer (20 to 80 years old) and matched control patients in 18 hospitals in six cities. Controls with tobacco-related diseases were excluded. Patients were interviewed between December 1980 and November 1982. Smoking data were obtained. Occupations were grouped either dichotomously as exposed to diesel exhaust (warehousemen, bus drivers, truck drivers, railroad workers, heavy equipment operators) or unexposed. Exposure categorization also conducted by NIOSH-based occupational classifications with job title classified as having "probable" exposure to diesel exhaust as either "high" (10 cases), "moderate" (16 cases) or "little or none" (476 cases). No significantly elevated risks were reported in this latter analysis (data not shown here). See also Boffetta *et al*., 1990. *Compared DE exposed to unexposed within each smoking category.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Boffetta *et al*. 1990 USA	Case- control Exposure by occupation: "Possible" exposure "Probable" exposure By duration: "Probable" DE 1-15 years 16-30 years 31+ years Truck driver* 1-15 years 16-30 years 30+ years Self-reported exposure: By duration 1-15 years 16-30 years 31+ years	 240 210 4 15 17 4 12 7 11 12 12	OR 0.92 0.95 0.52 0.70 1.49 1.83 0.94 1.17 1.21 0.90 1.04 2.39	 0.76-1.10 0.78-1.16 0.15-1.86 0.34-1.44 0.72-3.11 0.31-10.73 0.41-2.15 0.40-3.41 0.73-2.02 0.40-1.99 0.44-2.48 0.87-6.57	Study consisted of 2584 histologically confirmed lung cancer cases and 5009 controls derived from 18 hospitals in six cities. Controls were patients with current non-tobacco-related diseases matched by age, hospital and year of interview. Exposure was assessed by occupational titles and self-reported exposure to diesel exhaust. Results were adjusted for smoking, education and asbestos exposure by logistic regression. Occupations were classified as having probable, possible or no diesel exhaust exposure. Exposure prevalence was low. Only 15.6% of the controls were ever in an exposed job and 6.4% were considered probably exposed. Self-reported exposure to diesel exhaust had consistently higher point estimates of risk than those based on occupational classification, suggesting the possibility of recall bias. See also Hall and Wynder, 1984. *Duration of employment data only available for 23 cases and 27 controls of all patients classified as truck drivers (114 cases and 176 controls).
Damber and Larsson 1985 SWEDEN	Case-control By age of diagnosis: Professional drivers <70 years ≥70 years Truck drivers[#] <70 years ≥70 years By age & smoking status: Drivers/Nonsmokers** <70 years ≥70 years Drivers/Smokers** <70 years ≥70 years	 40 23 22 13 Not given Not given Not given Not given	OR 1.00* 3.15* 0.83* 5.70* 1.9 4.5 6.0 20.8	 0.66-1.50 1.66-6.00 0.50-1.40 2.22-14.67 0.5-5.5 1.1-16.4 3.5-10.3 9.4-46.0	Study included 604 male patients with lung cancer from the 3 most northern counties in Sweden (all new cases reported to the Swedish Cancer Registry in 1972 to 77 who had died at least one year before the start of the study in 1979). Matched controls were drawn from the national registry for causes of death. Living controls were also used. Data on occupational and smoking habits were obtained by questionnaire. Study focused on professional drivers, most of whose vehicles had diesel engines. Investigators noted that drivers had considerably higher average tobacco consumption than nondrivers. Authors stated that the study suggests a synergistic interaction between smoking and occupational exposure. See also Damber and Larsson 1987. Risk estimates presented for portion of cohort with date of birth after 1900. [#] Subset of all drivers. * Compared to nondrivers. ** Compared to nondrivers/nonsmokers, where "nonsmokers" included ex-smokers who had quit for at least 10 years.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Damber and Larsson 1987 SWEDEN	Case-control Professional drivers Years worked ≥1 ≥20 Adjusted for smoking ≥1 ≥20	 72 37 72 37	OR 1.3 1.5 1.0 1.2	 0.9-1.9 0.9-2.6 0.7-1.5 0.6-2.2	Study consisted of 600 men with lung cancer in northern Sweden reported to the Swedish Cancer Registry from 1972 through 1977 and dead before the start of the study (1979). Cases were matched with both dead and living controls. Results reported here are for comparisons with dead controls. Results with living controls were in good agreement. See Damber and Larsson (1985) for study focused on professional drivers only.
Boffetta *et al*. 1988 USA	Prospective Cohort Self-reported as DE: All DE exposed By duration exposure: 1-15 years 16+ years DE & smoking status*: nonsmokers ex-smokers current smokers Occupation: Railroad worker Truck driver Heavy equipment By occupation & DE: Truck/exposed Truck/nonexposed	 174 7 85 78 14 48 5 18** 18**	RR 1.18 1.05 1.21 1.73 11.06 19.82 1.59 1.24 2.60 1.22 1.19	 0.97-1.44 0.80-1.39 0.94-1.56[#] 0.60-4.95 6.27-19.53 11.20-35.07 0.94-2.69 0.93-1.66 1.12-6.06 0.77-1.95 0.74-1.89	Included 461,981 males, aged 40 to 79, participating in the American Cancer Society's Prospective Mortality Study in 1982. Follow-up for two years. Exposure assessment was based on self-reported (questionnaire) occupation and diesel exhaust exposure. Investigators stated that, although the sample was large, it was comprised of volunteers, who were healthier and were less frequently exposed to important risk factors such as smoking and alcohol. Reference population included men with no reported exposure or likely occupational exposure to diesel exhaust. Results were adjusted for smoking and other occupational exposures (asbestos, coal and stone dust, coal tar pitch, and gas exhaust). See Hall and Wynder, 1984. *Smoking data not available for all subjects. **Diesel exhaust exposure data not available for all truck drivers. [#]Test for trend reported by investigators as $0.05 < p < 0.10$.
Benhamou *et al*. 1988 FRANCE	Case-control Motor vehicle mechanic Transport equipment operators Professional drivers	 65 157 128	RR 1.06 1.35 1.42	 0.73-1.54 1.05-1.75 1.07-1.89	Study consisted of 1,334 histologically confirmed lung cancer cases and 2,409 controls matched on sex, age, hospital admission and interviewer. Study was conducted between 1976 and 1980. Results were adjusted for smoking and are limited to males. Occupation was determined by questionnaire (interview). The types of motor vehicle engines worked with were not specified. No evidence of increased risk with increased duration of exposure (years employed).

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Hayes *et al*. 1989 USA	Case-control Pooled Analysis Truck Drivers < 10 yrs employed ≥ 10 yrs employed Heavy Equipment < 10 yrs employed ≥ 10 yrs employed Bus Drivers < 10 yrs employed ≥ 10 yrs employed	 161 112 7 10 23 24	OR 1.0 1.5 1.5 2.1 1.1 1.7	 0.8-1.3 1.1-2.0 0.4-5.3 0.6-7.1 0.6-2.1 0.8-3.4	The study is a pooled analysis of three case-control studies conducted between 1976 and 1983 in Florida, New Jersey, and Louisiana. Total eligible cases = 2,291 and controls = 2,570. All occupational data were recoded from original interviews. No specific information regarding diesel exposure or engine type. ORs were adjusted for birth cohort (<1910, 1910-19, 1920-29, 1930+), usual daily cigarette use, and state.
Steenland *et al*. 1990 USA	Case-control Occupation data: 1)Teamster records data Long-haul driver Short-haul driver 2)Next-of-kin data Truck driver, diesel Truck driver, gasoline Truck driver, both Duration employment after 1959*: 1) Teamster records data Long-haul driver 1-11 years 12-17 years ≥18 years 2) Next-of-kin data Diesel truck driver 1-24 years 25-34 years ≥35 years	 162 228 213 48 72 56	OR 1.27 1.31 1.42 1.22 1.25 1.08 1.41 1.55 1.27 1.26 1.89	 0.83-1.93 0.81-2.11 0.89-2.26 0.79-1.88 0.81-1.95 0.68-1.70 0.90-2.21 0.97-2.47 0.70-2.27 0.74-2.16 1.04-3.42	Study consisted of 1,086 lung cancer cases and 1,085 controls among truck drivers in the Central States Teamsters Union. Information on work history was obtained from next of kin and union records. Subjects died in 1982-83 after applying for pensions, which required at least 20 years of union membership. Subjects were classified according to the job category in which they worked the longest. Union data provided no information on the type of truck driven. 90% of union long-haul drivers were also indentified as diesel truck drivers by next of kin. Results were adjusted for smoking and asbestos exposure. Smoking data obtained by next-of-kin interview used in both types of exposure classification. Steenland *et al*. (1992) summarized results from a recent industrial hygiene survey of exposure to diesel exhaust in the trucking industry, and found that elemental carbon measurements were generally consistent with the results; i.e., mechanics had the highest exposure and the highest risks, followed by long-haul and local drivers. Authors noted that exposure to asbestos may account for some of the observed effects in mechanics, but its confounding effect was probably small. Study results for truck mechanics and dock workers were elevated but not significant. *Study also presented risk estimates for duration of employment inclusive of the pre-1959 work era for both job ascertainment categories and for majority of job classifications.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Burns and Swanson 1991 USA	Case-control		OR		Occupational and smoking histories were obtained by telephone interview for 5,935 incident lung cancer cases and 3,956 incident colon and rectal cancer controls diagnosed between 1984 and 1987 and reported to the Detroit cancer registry. The smoking- and race-adjusted OR for all drivers (238 cases, 86 controls) was 1.88 (95% C.I. = 1.37-2.58), while drivers of "heavy trucks" (166 cases, 48 controls), maintained a higher risk even after adjustment for smoking, OR = 2.31 (95% C.I. = 1.56-3.42). Mechanics also had a significantly elevated OR for lung cancer (OR = 1.72, 95% C.I. = 1.15-2.59). The types of the vehicle engines were not specified. Results were adjusted for smoking. See Swanson *et al*. 1993.
	Drivers (white)	187	2.40	1.65-3.48	
	All drivers (race adj.)	238	1.88	1.37-2.58	
	Railroad workers	14	1.27	0.45-3.53	
Swanson *et al*. 1993 USA	Case-control		OR		Cases and controls were from OCISS (see Burns and Swanson, 1991 for description of subjects). Incident lung cancer cases among black and white males, aged 40 to 84, from 1984 through 1987 are included in this report. Controls were colon and rectal cancer cases. Information on occupation, smoking, medical history were obtained by telephone interview. Results were adjusted for age at diagnosis, race and smoking. *Test for trend $p \leq 0.05$.
	Occupation & duration:				
	1) White males				
	Heavy truck drivers				
	0 years	88	1.0	Reference	
	1-9 years	78	1.4	0.8-2.4*	
	10-19 years	38	1.6	0.8-3.5*	
	20+ years	121	2.5	1.1-4.4*	
	Light truck drivers				
	0 years	88	1.0	Reference	
	1-9 years	46	1.7	0.9-3.3	
	10+ years	36	2.1	0.9-4.6	
	Railroad workers				
	0 years	73	1.0	Reference	
	1-9 years	27	1.2	0.5-2.7	
	10+ years	40	2.4	1.1-5.1	
	2) Black males				
	Heavy truck drivers				
	0 years	12	1.0	Reference	
	1-9 years	27	2.7	0.8-9.2	
	10-19 years	16	1.9	0.5-7.2	
	20+ years	16	2.1	0.5-9.2	
	Railroad workers				
	0 years	15	1.0	Reference	
	1-9 years	22	2.6	0.8-7.9	
	10+ years	9	2.7	0.6-12.1	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Rafnsson and Gunnarsdottir, 1991 ICELAND	Cohort Truck drivers Duration employment: <2 years 2-10 years 11-30 years >30 years	 24 	SMR 2.14 2.70 2.46 0.68 2.32	 1.37-3.18 0.74-6.92 0.99-5.08 0.01-3.76 0.85-5.04	Cohort consisted of truck and taxi drivers in Reykjavik followed from 1951 to 1988. National mortality rates were used as for comparison. Information on truck drivers was obtained from their union. No data on smoking or type of vehicle engines used. No trend of increased risk with increased follow-up time was observed.
Guberan *et al*. 1992 SWITZERLAND	Cohort Professional drivers	 77	SMR 1.50	 1.23-1.81	Cohort identified from vehicle license records of professional drivers required to obtain special license during the period from 1949 to 1961. Excluding individuals born prior to 1900, 1,726 drivers were eligible. Lung cancer cases identified from death and tumor registries through 1986. No smoking data obtained. Approximately 1/3 to 1/4 of professional drivers were reported to be long-haul truck drivers. Death rates compared to regional mortality rates. A significant ($p<0.02$) upward trend in lung cancer mortality with time from first exposure was also observed: SMRs = 0.67, 1.18, 1.30, 1.35, and 2.59 for 0-14, 15-24, 25-34, 35-44, and ≥ 45 years, respectively (no confidence intervals reported).
Hansen 1993 DENMARK	Cohort Age on Nov. 9 1970 15-29 30-39 40-44 45-49 50-54 55-59 60-64 65-74 Total	 0 3 3 11 12 19 22 6 76	SMR 1.96 0.56 1.17 1.10 2.29 2.27 2.60 1.60	 0.40-5.73 0.12-1.64 0.58-2.09 0.57-1.93 1.38-3.58 1.42-3.44 0.95-5.65 1.26-2.00	Cohort consisted of 14,225 truck drivers followed for a 10-year period. Comparisons were made with another cohort of unskilled laborers. Members of the cohort were identified from the file of a nationwide census conducted in 1970. Self-reported occupation, trade, industry and employment on the day of the census were recorded. The study was comprised of unskilled male laborers 15 to 74 years old who were occupationally active on the day of the census. 627 truck drivers and 3,811 members of the control cohort died within the 10 years. No data on smoking. Diesel engines have comprised most of Danish fleet of trucks since the late 1940s.
Pfluger and Minder, 1994 SWITZERLAND	Case-control Professional drivers - smoking adjusted	 284 	OR 2.27 1.48	 1.99-2.58 1.30-1.68	Mortality of Swiss professional drivers (truck, bus and taxi drivers) was determined from death certificates and compared to census data to obtain occupation and age-specific death rates. No individual smoking data were available, but an indirect adjustment was conducted based on occupation specific mortality rates.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies among Transport (i.e., bus) and Equipment Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Raffle 1957 ENGLAND	Cohort Overall Bus & trolley drivers Age 55-64	 96 30	SMR 1.4	 N.S.	Cohort consisted of deaths, retirements and transfers due to lung cancer in London transport employees (bus and trolley workers, bus engineers), aged 45 to 64 years, in jobs with presumably different exposures to exhaust fumes in 1950 to 1954. Only cases arising during exposure employment were considered. Rates were compared to lung cancer mortality in other company employees. Diesel buses had been gradually introduced since the 1930s. At the end of WWII only 15% of the buses still used petrol. All had been replaced by 1950. Consequently, the duration of exposure of some workers to DE might have been short. No data on smoking. See also Waller 1981.
Waller 1981 ENGLAND	Cohort All workers Bus drivers Bus conductors Engineers, garages Engineers, central works Motormen and guards	 667 259 130 177 42 59	SMR 0.79 0.75 0.75 0.90 0.66 0.87	 Not presented. Not presented. Not presented. Not presented. Not presented. Not presented.	Cohort consisted of lung cancer deaths and retirements or transfers due to lung cancer in men, aged 45 to 64, employed within five categories of London Transport employees. Mortality was compared to men in Greater London. The study covered 25 years ending in 1974, thus including some of the data described by Raffle (1957). No data on smoking. Those who retired at age 65 or left earlier were not followed up, thus limiting the extent of case ascertainment.
Rushton *et al*. 1983 ENGLAND	Cohort	 102	SMR 1.01	 p=0.94	Cohort consisted of 8,684 men employed as maintenance workers in 71 bus garages in London for at least one year from 1967 to 1975. Follow-up through 1975. No data on smoking. Authors noted short follow-up period (average of 6 years). Lung cancer mortality was compared with the male population of England and Wales. The all-cause mortality was significantly lower than expected based on London residence.
Buiatti *et al*. 1985 ITALY	Case-control Transportation Taxi driving Train conductors	 45 20 7	OR 1.1 1.8 1.4	 0.7-1.6 1.0-3.4 0.5-3.9	Study consisted of 340 confirmed cases in males (and 817 controls) in Florence, diagnosed from 1981 through 1983 in the regional general hospital and a referral center for lung cancer. Controls were matched on sex, age, date of admission and smoking, and were from the same hospital. Diesel exhaust exposure was assessed by questionnaire for all jobs held for more than one year.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies among Transport (i.e. bus) and Equipment Workers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Wong *et al*. 1985 USA	Cohort Total By Duration <5 years 5-9 years 10-14 years 15-19 years ≥20 years All retired members Normal retired members	 309 10 25 53 58 163 155 86	SMR 0.99 0.45 0.75 1.08 1.02 1.07 1.64* 1.30**	 0.88-1.10 N.S. N.S. N.S. N.S. p=0.05 p<0.01 p<0.05	Cohort consisted of 34,156 male members of a heavy construction equipment operators union for at least one year from 1964 through 1978. Mortality experience was compared with that of the US white male population. Partial work history was available for some cohort members through the union. A random sample of union members was surveyed to determine smoking habits, and no significant difference between members and the general population was found. Work groups evaluated were considered to have high exposure to diesel exhaust (scraper operator, dozer operator, backhoe operator and loader operator) or low exposure (mechanical maintenance workers and engineers). Overall mortality in the cohort was less than that in the U.S. male population (SMR 0.81, 95% C.I. 0.79-0.84). Workers were also categorized by job title and potential exposure, but no significant risks were observed. Analysis of retirees found an excess risk for lung cancer* and emphysema. *Includes also retirements due to ill health. *Normal retirees are those workers retired at or over 65 and early retirees who reached 65.
Edling *et al*. 1987 SWEDEN	Cohort Bus company employees Bus drivers Bus garage workers Clerks	 6 5 1 0	SMR 0.67 0.69	Not presented	Cohort consisted of 694 bus garage employees followed from 1951 through 1983. Men were divided into three exposure categories (clerks, bus drivers and bus garage workers). Clerks were assumed to have had the lowest exposure to diesel exhaust and bus garage workers the highest. Authors stated that the power of the study to detect specific cancers was limited. No data on smoking.
Netterstrom 1988 DENMARK	Cohort Bus drivers	 15	SMR 0.87	 0.48-1.43	Cohort of 2,465 Danish bus drivers from three companies during the period 1978 to 1984. Cases were identified through death and cancer . registries. Death rates were compared with national rates. No data on smoking were available. Mean value for employment duration among the lung cancer cases was 30 years

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies among Transport (i.e. bus) and Equipment Workers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Gustavsson *et al*.	Cohort		SMR		Cohort consisted of 695 bus garage workers employed as mechanics,
1990	Total (deaths)	17	1.22	0.71-1.96	servicemen or hostlers for at least six months in five bus garages in
SWEDEN	DE exposure index:				Stockholm between 1945 and 1970. A nested case-control study was
	0-10*	5	0.97		performed within the cohort. Follow-up was through 1986. No data on
	10-30	5	1.52		smoking although no large variation in smoking habits was expected
	>30	7	1.27		within the cohort. Exposure to diesel exhaust and asbestos were
					assessed based on time period-specific data on job tasks. Lung cancer
	Nested case-control		RR		cases were identified through tumor and death registries. In the cohort
	(20 incident cases)				analysis regional rates were used for comparison.
	0-10*	5	1.0	Reference	
	10-20	2	1.34	1.09-1.64	*Cumulative exposure index values (unitless).
	20-30	3	1.81	1.20-2.71	
	>30	10	2.43	1.32-4.47	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies among Dock Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Gustafsson *et al*. 1986 SWEDEN	Cohort		SMR		Cohort consisted of 6,071 Swedish dockworkers first employed before 1974 for at least six months. The group was followed from January 1961 through January 1981. Cancer morbidity was determined among 6,071 dockworkers who had been alive and without cancer in January 1961. Comparison group was Swedish male population. Diesel trucks were introduced into Swedish ports in the late 1950s and became prevalent during the 1960s. No data on smoking. See Emmelin *et al*. (1993) for results from the follow-up study. Employment as a dockworker was the only information on diesel exhaust exposure used in the analysis.
	Deaths	71	1.29	1.02-1.63	
			SIR		
	Incident cases	89	1.53	1.24-1.80	
Emmelin *et al*. 1993 SWEDEN	Case-control				Study was a nested case-control of lung cancer among Swedish male dockworkers in the cohort studied by Gustafsson *et al*. (1986). 154 referents were matched to 50 cases on port and date of birth. Indices of exposure to diesel exposure were derived from employment records and records of annual fuel consumption by diesel vehicles. Three differenct exposure classifications were created: "machine time", "fuel consumption" and "exposed time". Information on smoking was obtained from questionnaires and interviews with foremen or workers who had worked with subjects. Response rate for mailed questionnaires was low (67%) but information from the interviews was available for 95% of the subjects. Some ex-smokers were classified as never smokers. No exposure level ("low", "medium", or "high") was significant for any DE exposure scheme (only "high" strata reported here). Comparisons based on exposure and smoking tended to find more elevated risks. Investigators noted that the increase in the OR for both smoking and exhaust exposure indicate that smoking does not explain the results from the exposure-only models, and that there may be an interaction between smoking and exhaust exposure. No information on asbestos exposure, which was said to have decreased by the 1970s. See also Gustafsson *et al*. (1986). * "Low" exposure category used for reference comparison. **Note: authors reported confidence intervals at 90% level.
	<u>Exposure variable:</u>		OR		
	Machine time				
	high*	14	1.3	0.3-5.6**	
	Fuel consumption				
	high*	15	1.7	0.5-5.9**	
	Exposed time				
	high*	19	2.9	0.8-10.7**	
	<u>Exposure & Smoking:</u>				
	Machine time				
	medium		1.8	0.5-6.6**	
	high		2.9	0.6-14.4**	
	smoker		5.7	2.4-13.3**	
	Fuel consumption				
	medium		1.5	0.5-4.8**	
	high		2.9	0.7-11.5**	
	smoker		5.5	2.4-12.7**	
	Exposed time				
	medium		2.7	0.6-11.3**	
	high		6.8	1.3-34.9**	
	smoker		6.2	2.6-14.6**	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies among railroad workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Kaplan 1959 USA	Cohort Total Most likely exposed	 154 49	SMR 0.80 0.875	 0.68-0.94 N.S.	Cohort consisted of 6,506 deaths among railroad workers from the Baltimore and Ohio Railroad Relief Department between 1953 and 1958. Subjects were categorized into 3 groups by exposed to diesel exhaust and compared with national lung cancer mortality rates. IARC noted that since the changeover to diesel engines began in 1935 and was 95% completed by 1959 (Garshick *et al*. 1988), few, if any, of the lung cancer deaths could have occurred in workers with more than 10 years of exposure to diesel diesel exhaust. No data on smoking.
Howe *et al*. 1983 CANADA	Cohort Entire cohort Retired after 1950 Exposure to DE "nonexposed" "possibly" exposed "probably" exposed	 933 897 239 407 279	SMR 1.06 1.00 1.20 1.35	 0.99-1.13 p=0.13 p<0.001	Study consisted of 43,826 males of the Canadian National Railway Co. retired and alive in 1965 and followed until 1977. No data on smoking. However, authors note that this may not be crucial since conclusions were based on internal comparisons where no large variation in smoking habits was likely. It was also noted that certain smoking-related deaths were elevated. The results remained unchanged when individuals likely to have been exposed to asbestos were excluded from the analysis.
Garshick *et al*. 1987a USA	Case-control Age (years) ≤64 ≥65 DE Exposure: Diesel-years ≤ 64 worker 5-19 ≥20 Diesel-years > 65 worker 5-19 ≥20 Minus shopworkers* ≥20 years of exposure Years of cumulative DE exposure:** 5-14 ≥15	1256 335 921	OR 1.41 0.91 1.02 1.64 0.95 0.94 1.55 1.07 1.43	 1.06-1.88 0.71-1.17 0.72-1.4 1.18-2.2 0.79-1.13 0.56-1.59 1.09-2.21 0.69-1.66 1.06-1.94	Study consisted of Railroad Retirement Board registrants (1,256 cases and 2,385 matched controls) who died between March 1981 and February 1982. Subjects were active and retired workers with at least 10 years work experience. Persons who died from cancer, suicide, accidents or unknown causes were excluded as controls. Results were adjusted for smoking and asbestos exposure. The baseline study year was 1959, when diesel engines had nearly replaced all steam engines. Consequently, few of these workers were exposed to asbestos. Personal exposure was assessed by industrial hygiene sampling in 39 job categories. Job titles were used to dichotomize subjects into exposed and unexposed groups (Woskie *et al*. 1988a,b). See also Garshick *et al*. (1988). *Shopworkers had the highest levels of asbestos exposure. **These results excluded exposure occurring within 5 years before death. The shortest exposure category, 0 to 4 years, was used as a reference group.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Studies among railroad workers (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Garshick *et al*. 1988 USA	Cohort	1694	RR		Cohort consisted of 55,407 white male railroad workers aged 40-64 exposed to little or no asbestos who had started work between 1939 and 1949 and had worked 10 to 20 years after 1959. Follow-up through 1980. Industrial hygiene data were used to categorize jobs as exposed or unexposed. No data on smoking; however, authors noted that there was no difference in smoking habits by job title in comparison studies of current workers (see Garshick *et al*. 1987). Diesel exhaust exposure in the US railroad industry occurred after WWII. The approximate midpoint of dieselization was in 1952 and by 1959, 95% of the locomotives were diesel-powered. Workers aged 40 to 44 in 1959 were the group with the longest possible duration of exposure. Most workers with potential asbestos exposure were excluded, though some did have potential exposure to asbestos (shopworkers and hostlers). Analyses were done with and without these groups. Exposure was assessed from samples of respirable dust taken in 1980s (Woskie *et al*. 1988a). Mean exposure levels suggested a five-fold range of exposure between clerks and shopworkers (Woskie *et al*. 1988b). These values confirmed the assignment of categories of diesel exhaust exposure in the present study and Garshick *et al*. 1987. * Excluding exposure to diesel exhaust over the 4 years preceding the year of death
	By Age in 1959 w/ DE:				
	40-44		1.45	1.11-1.89	
	45-49		1.33	1.03-1.73	
	50-54		1.12	0.88-1.42	
	55-59		1.18	0.94-1.50	
	60-64		0.99	0.74-1.33	
	Minus those w/ asbestos exposure				
	40-44		1.57	1.19-2.06	
	45-49		1.34	1.02-1.76	
	By Years DE Exposure:*				
	1-4 years		1.20	1.01-1.44	
	5-9 years		1.24	1.06-1.44	
	10-14 years		1.32	1.13-1.56	
	≥ 15 years		1.72	1.27-2.33	
	Minus those w/ asbestos exposure				
	1-4 years		1.34	1.08-1.65	
	5-9 years		1.33	1.12-1.58	
	10-14 years		1.33	1.10-1.60	
	≥ 15 years		1.82	1.30-2.55	
Nokso-Koivisto and Pukkula, 1994 FINLAND	Cohort Total	236	SIR 0.86	0.75-0.97	Cohort consisted of 8,391 members of the Finnish Locomotive Drivers' Association from 1953 to 1991 (including retirees). Information was not available for 302 members. No smoking data were available. The overall incidence for all cancer sites was lower than expected when compared to national rates (SIR = 0.95).

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Additional Studies Other Than Those Listed In Above Categories

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Wegman and Peters, 1978, USA	Case-control Total study Transportation equipment operatives - Registry derived - Combination w/ registry data	 91 8 5	OR 8.67 1.26	 Not presented. Not presented.	Tumor registry-based study of oat cell carcinoma during 1965 to 1972. Cancer controls identified from same registry. Smoking data collected but not used in analysis (94% cases and 78% controls smoked). Two methods used to classify occupation, registry-derived or combination of registry and next-of-kin questionaire data. Number of cases classified as transportation equipment operatives decreased from 8 to 5 between two methods.
Coggon *et al*. 1984 ENGLAND	Case-control Total DE exposed High DE exposure	 172 32	RR 1.3 1.1	 1.0-1.6 0.7-1.8	Study included all men 40 years of age in England and Wales who had died of tracheobronchial cancer from 1975 through 1979. A job exposure matrix was constructed in which occupations were grouped according to likely exposure to each of nine known or putative carcinogens. Occupational information abstracted from the death certificates. No information on smoking. IARC noted the limitations of information on death certificates, the young age of the subjects, short exposure and latency times, and the lack of data on smoking and other potential confounders.
Lerchen *et al*. 1987 USA	Case-control Diesel exhaust fumes - adjusted for smoking Diesel engine mechanics - adjusted for smoking	 7 5	OR 0.6 1.0	 0.2-1.6 0.2-2.0	Population-based case-control study of 506 patients diagnosed between January 1980 and December 31, 1982, and reported to the New Mexico tumor registry (333 males and 173 females). Data on lifetime occupation and smoking were obtained by personal interview and self-reported history of exposure to specific agents. Matched controls were selected randomly from the telephone directory or for persons over 65 from the roster of participants in a health insurance plan. Only seven males reported exposure to diesel exhaust.
Magnani *et al*. 1988 ENGLAND	Cohort All DE exposure	 Not given	SMR 1.07	 1.04-1.10	General population-based cohort analysis of death certificate and census survey information on 31,925 men with lung cancer between 1970-72. No smoking data were available. A job-expousure matrix was developed for several potential carcinogens, including diesel exhaust.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

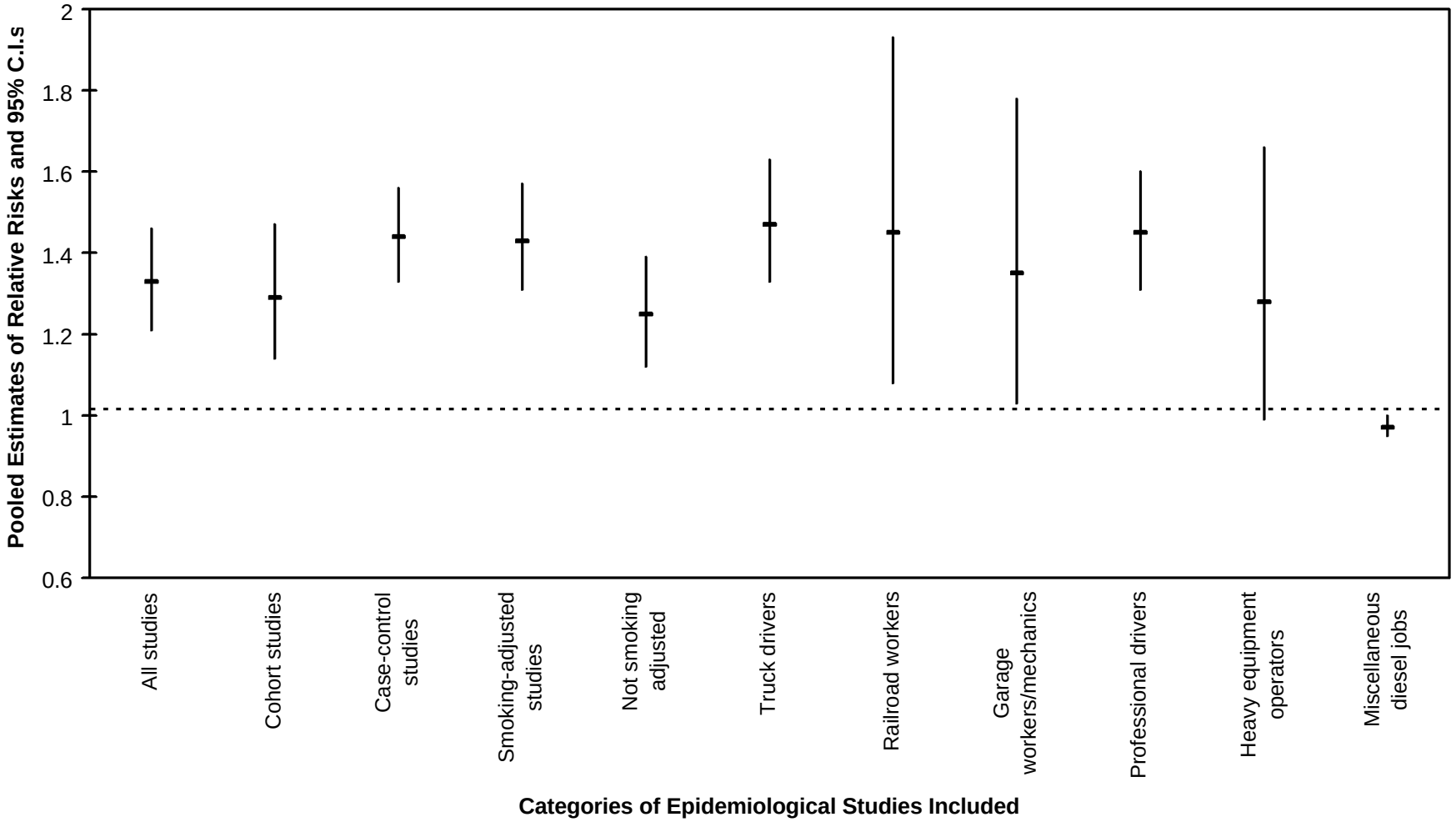
Table 6-5. Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer

Additional Studies Other Than Those Listed In Above Categories (Continued)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or P-Value	Comments
Siemiatycki *et al*. 1988 CANADA	Case-control Lung cell types among DE exposed: Oat cell Squamous cell Adenocarcinoma Other Total DE-exposed occupations minus mining:	 34 81 28 34 177 70	OR 1.1 1.2 0.9 1.0 1.1	 0.8-1.5** 1.0-1.5** 0.6-1.2** 0.8-1.4** 0.8-1.5**	This population-based case-control study provided information on the association between several cancer types and 10 types of exhaust and combustion products. Interviews were carried out for 3,726 cancer patients, aged 35 to 70, diagnosed in any of 19 participating Montreal area hospitals. Each type of cancer was a case series; reference groups were selected from among the other cancer patients interviewed. Results reported are adjusted for smoking, socioeconomic status, ethnic group and several other potential confounders. Authors noted that the excess lung cancers were concentrated among mine and quarry workers. **Authors reported 90% confidence intervals.
Bender *et al*. 1989 USA	Cohort State highway workers	 Not given	SMR 0.69	 0.52-0.90	Cohort consisted of Minnesota highway workers employed for a minimum of one year and working at least one day after January 1, 1945. Mortality was compared to state rates. No data were available on smoking. Overall mortality was significantly lower than the expected, SMR = 0.83 (95% C.I. = 0.73-0.94).
Kauppinen *et al*. 1993, FINLAND	Case-control Engine exhaust exposure: Any exposure ≥ 1 month 1 month - 5 years > 5 years	 8 5 3	 OR 1.7 0.39 2.21	 0.55-5.20** 0.05-2.94** 0.65-7.48**	Nested case-control study of woodworkers in Finland consisted of 136 lung cancer cases diagnosed between 1957 to 1982 and 408 matched controls. Original cohort consisted of 7,307 workers from 35 factories. Multiple chemical exposures were analyzed for, including engine exhaust (combination of diesel and gasoline engines). Smoking, age, and other chemical exposures were adjusted for; however, only a small number of individuals were categorized as having been exposed to engine exhaust. **Authors reported 90% confidence intervals.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or p-values reported in original study. DE = Diesel Exhaust
OR=Odds Ratio, RR= Relative Risk, SIR= Standardized Incidence Ratio, SMR= Standardized Mortality Ratio

Figure 6.2.1: Pooled Estimates of Relative Risk of Lung Cancer in Epidemiological Studies Involving Occupational Exposure to Diesel Exhaust (Random Effects Models)



7.0 QUANTITATIVE CANCER RISK ASSESSMENT

This chapter provides estimates of the risk of humans developing cancer due to the inhalation of diesel exhaust in the atmosphere. The first section introduces the mass of particles as the measure of the carcinogenic agent in diesel exhaust to be used in these analyses. The second section describes the use of occupational studies to predict human risks due to exposures that occur in the general environment. The final section brings together these results and proposes a range of values for the upper confidence limits (UCL) for unit risk to be used for risk assessment.

Epidemiological data are available upon which one can base risk estimates for humans. While there are issues surrounding the quantitation of worker exposure to diesel exhaust, the uncertainty of extrapolating from one species (rat) to another (human) is avoided by using the epidemiological data to estimate risk to humans from diesel exhaust exposure. OEHHA prefers, on balance, to use the epidemiological data in order to estimate risk to humans from diesel exhaust exposure.

7.1 MEASURE OF DIESEL EXHAUST AS A CARCINOGENIC AGENT

The complex and potentially variable mix of chemical species in the condensed phase and the vapor phase of diesel exhaust, requires the measure of exposure related to carcinogenic risk to be specified. The most commonly used measure of exposure is atmospheric concentration of particles in $\mu g/m^3$. That measure is obtained from the mass of particles collected on a filter per volume of the air that flowed through the filter. On the basis of its relation to health studies and its general practicality, that measure is used in this risk assessment.

7.2 HUMAN RISK ESTIMATES FROM EPIDEMIOLOGICAL STUDIES

This section considers several methods to establish the range of risk to the public from human occupational studies. The results of the meta-analysis provide information useful in bracketing the broadest likely range of plausible carcinogenic potencies for diesel exhaust. The pooled relative risk values derived from the 12 epidemiological studies in the meta-analysis which adjusted for smoking were 1.44 (95% C.I. 1.32 -1.56) for the fixed effects model and 1.43 (95% C.I. 1.31 - 1.57) for the random effects model. The magnitude of these relative risks provide information on the potential magnitude of the cancer risk associated with diesel exhaust exposure. For the random effects model the upper 95% confidence limit on excess relative risk is 0.57.

None of the studies in the meta-analysis provide direct measurements of exposure concentration over the time of their follow up. Therefore, to the extent that the meta-analysis can be used to bracket the carcinogenic potency of diesel exhaust, the exposures of the various study populations need to be reconstructed. Hammond (1998) has reviewed the available industrial hygiene survey literature on the occupations considered in the meta-analysis (bus garage workers, mechanics, truck drivers, heavy equipment operators, railroad workers) and provided estimates of the plausible possible ranges of workplace exposures of diesel exhaust respirable particulate matter for those occupations. Because of the overall limitations in the data, the

estimated ranges for each occupational subgroup of interest are particularly broad. The lowest plausible estimate of occupational exposure for any such subgroup is 5 $\mu g/m^3$ (heavy equipment operators). The highest plausible estimate of any occupational subgroup is 500 $\mu g/m^3$ (bus garage workers, railroad workers, mechanics). The total range of plausible exposures for the different populations therefore varies 100-fold. Using these air concentrations and the assumption that workers inhaling $10m^3$ of air per work shift were exposed to them for over 45 year period for a 70 year lifetime, it is possible to characterize a bracket of risks compatible with the results of the meta-analysis:

$$q_1^* = \frac{\text{Excess relative risk x CA lifetime lung cancer risk}}{\text{Air concentration x exposure factor x intermittency factors x duration of exposure/lifetime}}$$

$$= \frac{0.57 \times 0.025}{(5 \text{ or } 500\ \mu g/m^3) \times 10\ m^3/\text{shift}/20m^3/d \times 5d/7d \times 48wk/52wk \times 45\ yrs/70yrs}$$

Therefore, the results of the meta-analysis bracket lung cancer risks up to approximately $1.3 \times 10^{-4}\ (\mu g/m^3)^{-1}$ (assuming all the worker populations in the meta-analysis were exposed to 5 $\mu g/m^3$) to $1.3 \times 10^{-2}\ (\mu g/m^3)^{-1}$ (assuming all the workers populations in the meta-analysis were exposed to 500 $\mu g/m^3$). As these assumptions establish the extreme bounds of probable exposures, and such calculations based upon a meta-analysis are novel and subject to further possible refinements, these results are not incorporated into the range of risks. However, these results do bracket the carcinogenic potencies which would be consistent with the results of the meta-analysis and the broadest range of exposure estimates.

A more plausible range can be estimated by determining the 90% confidence interval (CI) of the range of risks. For the meta-analysis the range of concentrations thought to be plausible by Hammond (personal communication) was 5 to 500 $\mu g/m^3$ with a mean of about 200 $\mu g/m^3$, which corresponds to a unit risk of $3.3 \times 10^{-4}\ (\mu g/m^3)^{-1}$. Using that concentration range as the 98% CI for a shifted lognormal distribution fixes the geometric standard deviation at 1.22 with a shift of the origin of the distribution by 330 $\mu g/m^3$. The 90% CI for this distribution of concentration is [52.5 to 356.5 $\mu g/m^3$], corresponding to a 90% CI for the distribution of unit risk of [1.6×10^{-4} to $1.2 \times 10^{-3}\ (\mu g/m^3)^{-1}$].

In this section, OEHHA focuses upon the railroad worker studies as being particularly useful in developing the range of unit cancer risks. This section develops quantitative relationships between lung cancer risk and exposure to diesel exhaust for two nation-wide studies of lung cancer rates in U. S. railroad workers. These relationships provide additional values for the range of risk to the general California population. The first, Garshick *et al*. (1987a), is a case-control study. Using a logistic regression, that study determined the coefficient of the logistic relationship of the odds of lung cancer for duration of the workers' exposure to diesel exhaust. The coefficient determined in that study is used below to estimate lifetime unit risks for exposure of the general population. The second study, Garshick *et al*. (1988), is a cohort study. Using a proportional hazards model, that study calculated the relative hazard of lung cancer for increasing duration of worker exposure. However, those numerical results have not been

supported by Garshick (1991); so instead of using them to derive lifetime unit risks for the general population, new analyses are performed with the individual data, upon which that study is based, to determine a linear relationship of lung cancer hazard for worker exposure to diesel exhaust.

The term hazard, as used here, is for a prediction of incidence (cancers per year per population) resulting from a model. Relative hazard is generally called relative risk in epidemiological model work, and the term, relative risk, will be used below in the context of the epidemiology results. The lifetime inhalation unit risk, often simply called unit risk, is defined as the probability of contracting lung cancer from a 70-year exposure to a unit concentration (1 $\mu g/m^3$) of diesel exhaust.

The unit risks ultimately derived for the general population assume that the mass concentration of particles governs the risk of diesel exhaust, regardless of the particular type of diesel engine or fuel. The resulting estimate of risk entails uncertainties due primarily to the limited exposure information available and to the choice of models and data used in the analysis.

These two studies are among a number of studies establishing excess relative risk of lung cancer among workers exposed to diesel exhaust, as described in Section 6.2 above. These two studies are specifically selected for the quantitative risk assessment because of their general excellence, their apparent finding of a relationship of cancer rate to duration of exposure and because of the availability of measurements of diesel exhaust among such railroad workers from the early 1980's in other studies. The case-control study appears to have an advantage in obtaining direct information on smoking rates, while the cohort study has an advantage of smaller confidence intervals of the risk estimates.

7.2.1 ESTIMATING CUMULATIVE EXPOSURE

The risk relationships developed below use cumulative atmospheric exposure to diesel exhaust particles as the effective dose. The use of cumulative exposure, defined as the area under the curve (AUC) of concentration versus time, requires a specification of the temporal pattern of exposure concentration. Yet direct measurements of exposure concentration over the time of the follow up are not available.

Thus, the current calculations require reconstruction of the exposure history in order to determine cumulative exposure. The reconstruction is undertaken using (1) personal exposure measurements on railroad workers just after the end of the follow-up period in that study, (2) historical data on the dieselization of locomotives in the United States, and (3) descriptive information. The analysis below includes workers on trains and excludes shop workers from the original cohort.

7.2.1.1 EXPOSURE MEASUREMENTS IN THE EARLY 1980s

Woskie *et al*. (1988b) estimated national average concentrations of respirable particulate matter

(RSP) for 13 job-groups. These concentrations were obtained by temperature correction of measurements of respirable particulate matter (RSP) made in 1982-1983 in the northern region of the United States, as reported in Woskie *et al*. (1988a). The investigators adjusted these concentrations to remove the portion of RSP attributable to environmental tobacco smoke (ETS). The average values of the ETS-adjusted RSP for the principal categories of workers are compiled in Table 7-2 for exposed and unexposed workers.

7.2.1.2 RECONSTRUCTION OF THE TIME COURSE OF CONCENTRATION

In order to estimate the time course of the exposure factors for the cohort, it is necessary to make assumptions about time trends of nationwide average concentration breathed by the workers. The exposure measurements made just after the follow-up period constitute a baseline for the reconstruction. The reconstruction of the time course of concentration proceeds by developing an exposure factor to multiply these baseline values. The analyses below explore the effect of alternative patterns of exposure concentration and baseline values.

Dieselization of the U.S. railroads began after the Second World War ended in 1945. The exposure of the railroad workers up until 1981 can be divided into two periods: (1) an initial period of increasing dieselization of U.S. locomotives from 1945 until mostly completed in 1959 and (2) a subsequent period of a moderate rate of addition of locomotives that were less smoky.

Woskie *et al*. (1988b) reported data showing a linear rise of percent dieselization with time in the first period from 1945 to 1959. They reported data from the Bureau of Labor Statistics showing that by 1947 fourteen percent of locomotives were diesel, by 1952 fifty-five percent were diesel, and by 1959 ninety-five percent were diesel. This linear rise of dieselization may be expected to have produced a linear rise of the national average exposure concentration around the trains. This linear rise is used in all the more realistic exposure patterns.

The exposure of workers on trains would then generally have declined as the newer, less smoky locomotives replaced the older, smokier locomotives on the main lines. To quantify the anecdotal information of greater smokiness of locomotives in the period before 1960, the national average exposure concentration was assumed to decline linearly in the second period, 1960-1980, to the baseline measured in 1982-3. The decline assumed from 1959 to 1980 is consistent with the report of sharp decreases of emissions of new engines between the 1970's and the 1980's. Emissions from naturally aspirated four-stroke engines declined from 2.1-3.0 g/kW-hr in the 1970's to 0.25 -0.6 g/kW-hr in the 1980's (Sawyer and Johnson, 1995).

In order to bracket the exposure of the railroad workers to diesel exhaust a variety of patterns of exposure are considered. The patterns are characterized by two components: a) the extent of change from 1959 to 1980 in diesel exhaust exposure, expressed as a ratio, and b) the average exposure concentration for the workers on trains measured in the Woskie *et al*. (1988a) study (i.e., the baseline). The alternate ratios are as follows: a) a ratio of 1 suggested and used in Crump *et al*. (1991) as more realistic than the Garshick *et al*. (1987a, 1988) assumption of constant concentration from 1959-1980 and none before that; b) a ratio of 2 suggested by K. Hammond to allow for a modest peak in 1959; c) a ratio of 3 allowing for more peak, a scaled

down version of the exposure factor of 10 that Woskie *et al*. (1988b) reported for exposure concentration of shopworkers to nitrogen dioxide in enclosures including engine test sheds; and d) a ratio of 10, peak of the magnitude of values for the engine test sheds. The alternate baselines of exposure concentrations are as follows: 1) 40 $\mu g/m^3$, obtained by subtracting the background measurement of the unexposed workers from the measurement of the train workers, rounded down (See Table 7-2 footnotes: 82-39=43); 2) 50 $\mu g/m^3$, which also subtracted background from the train worker measurements but rounded up to allow somewhat for measurements of workers on trains not having as much exposure to non-diesel exhaust background particulate as the clerks; and 3) 80 $\mu g/m^3$, obtained by assuming that the entire ETS-adjusted RSP of the train workers is diesel exhaust while the clerks are considered unexposed to diesel exhaust (0 concentration).

The specific alternative patterns of linear decline (if any) of concentration from 1959 through 1980 are:

1) no decline, constant at the baseline values of 50, a ramp (1,50) pattern suggested and used in Crump *et al*. (1991) and subsequently by Crump (1995, 1996a,b, 1997).
2) declining 3-fold from a peak of 150 to a baseline of 50, a roof (3,50) pattern, the preferred pattern in this report;
3) declining 10-fold from a peak of 500 to a baseline of 50, a roof (10,50) pattern, suggested in information submitted by the Engine Manufacturers Association;
4) declining, 2-fold from a peak of 80 to a baseline of 40, a roof (2,40) pattern suggested by K. Hammond, one of the investigators in the Woskie *et al*. study; and
5) declining 3-fold from a peak of 240 to a baseline of 80, a roof (3,80) pattern, a variant on Pattern 3 for not subtracting background ETS-adjusted RSP in the exposed group while still maintaining unexposed workers at zero concentration.

Figure 7-4 presents the block, ramp, and roof exposure pattern models.

7.2.1.3 CALCULATION OF CUMULATIVE EXPOSURE

The estimate of the time course permits calculation of the overall average cumulative exposure for the cohort for each year of the follow-up period, 1959-1980. See Figure 7-1 as an example of calculating the cumulative exposure factor using the roof pattern for the categories of duration of exposure originally selected by Garshick *et al*. (1988). The cumulative exposure factor is calculated as the area under the curve (AUC) of the exposure factor (EF, ratio of concentration to baseline concentration) for successive years. Cumulative exposure is the cumulative exposure factor times the baseline value.

7.2.1.4 INTERMITTENCY CORRECTION

The equivalent exposure duration for non-continuous exposure is scaled on the basis of volume of air breathed. Exposure durations are calculated to have the same cumulative yearly intake of the substance as produced by continuous inhalation of 20 m^3/day at the concentration of the substance breathed in. Assuming that the average exposed member of the cohort inhales 10 m^3 during an 8-hour working day implies an adjustment factor of 10/20 to multiply the exposure

concentration to account for ventilation rate not equaling the standard human daily inhalation of 20 m^3/day. Adjusting for the discontinuous work week and work year yields additional adjustment factors of 5/7 for exposure days per week and 48/52 for weeks per year, all to multiply the exposure measure. In order to take account of the non-continuous work exposure, the resulting overall multiplicative factor on exposure duration is

$$(10/20)(5/7)(48/52) = 0.33.$$

7.2.2 DETERMINING LIFETIME UNIT RISK FROM THE RELATIVE-RISK SLOPE

The analyses below calculate the relationship between relative risk (relative hazard) and duration of exposure. The relative risk is the prediction of the ratio: incidence (yearly death rate per population) of lung cancer due to diesel exhaust divided by the background incidence of lung cancer. In the principal modeling of both sets of epidemiological data, reported below in this chapter, relative risks are fitted linearly to duration of exposure. From that slope, an estimate of the slope with respect to cumulative exposure for the specific alternative patterns of occupational exposure considered is obtained by modifying the duration scale for the slope. The approximation for this modification is simply to multiply the duration scale by the overall area under the curve (AUC) of the desired pattern and to divide by the total duration of exposure in the analysis.

Approximations may often be used to determine lifetime unit risk from this slope, but the present work will, for consistency and accuracy, use life-table calculations for that determination. This calculation starts with a background life table for lung cancer in California (Table D-1). For each unit risk to be calculated, a modification of that table is constructed in a way that includes the predicted effect of a lifetime exposure to 1 unit of concentration, 1 $\mu g/m^3$ in the present calculations. The predicted effect is incorporated by multiplying the background lung cancer incidence for each age interval in the table by the relative risk (relative hazard) for that age interval. See, for example, Table 7-1. The relative risk is (1+ excess relative risk due to exposure). The excess relative risk due to exposure for unit concentration is the slope of relative risk with concentration, obtained from the epidemiological analyses. Using the general model based on cumulative exposure, as in the present calculations, the excess relative risk requires the slope coefficient per concentration-yr to be multiplied by the age in years for each age group in the table and to be divided by the intermittency factor. Any ages that fall within the number of years of detection lag prior to the target age have zero excess relative hazard. The modified table is completed in the manner of the original table. The lifetime unit risk is then the following difference: the probability of lung cancer at the target age in the table modified by exposure less the probability at the same age in the original table.

7.2.3 USE OF THE GARSHICK *ET AL.* (1987a) CASE-CONTROL STUDY TO ESTIMATE UNIT RISK

The first study used to estimate lung cancer risk due to diesel exhaust exposure is the case

control study of U. S. railroad workers by Garshick et al. (1987a). For this case-control study Garshick *et al*. (1987a) collected 15,059 US railroad worker death records for 1981. They matched each of 1256 lung-cancer cases with 2 other deaths, each of those having nearly the same date of birth and death. For each of the controls, death was due to a specified natural cause with no mention of cancer on the death certificate. For each subject, Garshick *et al*. (1987a) determined years in a job with diesel exposure, asbestos exposure and smoking history. Taking into account the effect of age, their analysis used multivariate conditional logistic regression to determine the relationship between lung cancer and duration of exposure to diesel exhaust. For workers with more than 20 years exposure and for exclusion of shopworkers, they calculated the odds ratio was 1.55 (95% CI = 1.09, 2.21) with a referent category of 0 to 4 yr work in a job exposed to diesel exhaust.

From the odds ratio for 20 yr duration of exposure, the coefficient of increase with duration of exposure was estimated by assuming a linear rise over the 20 yrs. Using a calculation similar to that used by Garshick *et al*. with shopworkers included, the slope coefficient for the odds ratio is 0.022 (90% C.I. = 0.0071, 0.037) yr^{-1}. Because the odds ratio approximates relative risk (Breslow and Day 1980, pp. 69-73), this value is approximately the rate of increase of relative risk (relative hazard) and is used in a life table to obtain the lifetime unit risk. The modified life table calculation for unit concentration (1 $\mu g/m^3$) for 5-yr. lag from carcinogenesis to death is in Table 7-1. The resulting unit risks are presented in Point I in Table 7-3. The highest values in that set are for the assumption that workers on trains have a ramp (1,50) pattern of exposure. The 95% UCL for lifetime unit risk is 2.4×10^{-3} $(\mu g/m^3)^{-1}$, with an MLE of 1.4×10^{-3} $(\mu g/m^3)^{-1}$. For the roof (3,50) pattern of exposure, the procedure is similar, but the exposure scale is increased by the ratio 65/22, representing the ratio of area under the EF of the roof to the area under the EF of the block. The resulting 95% UCL for lifetime unit risk is 1.0×10^{-3} $(\mu g/m^3)^{-1}$, with an MLE of 6.2×10^{-4} $(\mu g/m^3)^{-1}$. The lowest values in the set are for the roof (10,50) pattern of exposure. Using a similar approach, multiplying the exposure scale by the AUC ratio of 191/22, the 95% UCL for lifetime unit risk is 3.6×10^{-4} $(\mu g/m^3)^{-1}$, with an MLE of 2.1×10^{-4} $(\mu g/m^3)^{-1}$.

Using the slope coefficient for the analysis including shopworkers, reported in Garshick *et al*. (1987a), McClellan *et al*.(1989) previously calculated the expected increase in U.S. lung cancer deaths per year for each $\mu g/m^3$ of diesel exhaust exposure for two alternative exposure concentrations, 125 $\mu g/m^3$ and 500 $\mu g/m^3$, constant from 1959-1980. Mauderly (1992a) used these death rates to estimate unit risks, finding expected values of 1.2×10^{-3} (lifetime-$\mu g/m^3)^{-1}$ and 2.9×10^{-4} (lifetime $\mu g/m^3)^{-1}$, respectively. These values are close to the higher MLE values just given. Even though the higher concentrations assumed by McClellan *et al*. would tend to produce lower unit risks, the effect of using the more accurate life table method has a counteracting effect.

7.2.4 USE OF THE GARSHICK *ET AL*. (1988) COHORT STUDY TO ESTIMATE UNIT RISK

The second study selected to estimate lung cancer risk due to diesel exhaust exposure is the retrospective cohort study of U. S. railroad workers by Garshick *et al*. (1988). The present

analysis uses the individual data collected for that study in new calculations to determine slopes for the relationship of incidence to cumulative exposure. The analysis uses reconstructions of exposure, the ramp and the roof exposure patterns, to adjust the slope obtained from the analysis that is implemented with duration of exposure as the measure of exposure.

Further material on the cohort is developed in Appendices D, E, F. Appendix E contains references to correspondence cited in this chapter. (The original unpublished documents referred to in Appendix E are available on request from the California Air Resources Board, Stationary Source Division or from the US EPA docket for the Health Assessment Document for Diesel Emissions at the National Center for Environmental Assessment, Washington, DC. 20460 (1997)).

7.2.4.1 DESCRIPTION OF THE ORIGINAL STUDY

As described in more detail in Section 6.2, the cohort consisted of 55,407 railroad workers, who were aged 40-64 in 1959 and who had started railroad service 10-20 years earlier; 1694 lung cancers were identified. The unexposed group in the cohort, the clerks and signal tenders, constituted 25.3% of the whole cohort. To develop the original data set, Garshick *et al*. (1988) obtained the following information for each individual in their cohort of railroad workers for the follow-up years of 1959-1980: cause of death by death certificate, the primary job classification for each year, and months worked in that classification in each year. In addition, the investigation obtained the age at the start of follow-up in 1959, total service months and, for those workers who began work after 1946, the date of starting work. From these data Garshick *et al*. calculated the elapsed time of exposure for each individual from 1959 up to each follow-up year or up to the four years before each follow-up year.

7.2.4.2 THE CURRENT APPROACH

Because of much uncertainty about the proportion of shop workers exposed to diesel exhaust, the present work excludes them from the analysis, as suggested by the study authors and other participants at the Diesel Exhaust Workshop, January, 1996. Garshick (1991) had previously called attention to dilution of the effect of diesel exhaust on the shop workers because of the inclusion of shopworkers in that cohort who had no true exposure. The original study obtained risk estimates both with and without the shop workers, and found the results changed very little. The exclusion of shop workers simplifies the analysis in that lung burden calculations are not needed because the exposures of other exposed workers, namely train workers, are sufficiently low that lung burden may be assumed essentially proportional to atmospheric exposures.

Exposure measurements for 1982-83 (Woskie *et al*. 1988a), just after the end of the follow-up period, show that train workers considered here all experienced approximately the same average concentration of diesel exhaust (for example, 50 $\mu g/m^3$, rounded, for use in determining unit risk in this work). The present work uses years with any month of exposure time, excluding the four years previous to each year of observation as the average lag time from carcinogenesis to death. This calculation of exposure time starts in 1952 and continues yearly through 1980, the end of

follow-up. It extends 7 years back from 1959, the start of follow-up, to account on the average for the assumed linear rise of exposure from 1945 to 1959. The unexposed workers are assigned zero exposure time throughout.

The current analysis uses two programs in the EPICURE software package, which is designed for several standard kinds of epidemiological analysis. The first program, DATAB, reduces the individual data to cells with each desired variable having a single value for the cell. The cells are designated by a set of numbers, one for each categorical variable to determine the category number of that variable. The second program, AMFIT, determines parameters of a model to provide a best fit of the data using Poisson regression, a maximum likelihood procedure (Breslow and Day, 1987). The calculation approach is described in more detail for the closely related calculations using general models, in Appendix D.

The assumptions not otherwise specified here are essentially those of Garshick *et al*. (1988). For example, all years of the study are included, and their rather irregular boundary points on years of exposure are used. See Figure 7-1.

7.2.4.3 RESULTS

The current analysis explored the fit and other characteristics of a number of forms of a general model. The model that appears to be most satisfactory is the one with linear and quadratic continuous covariates, age and calendar year. The slope calculated for relative risk (relative hazard) per year of exposure is 0.015 (95% CI: 0.0086 to 0.022) yr^{-1}. Figure 7-2 shows a comparison of the trend of relative risk given by this slope and the trend given by category of exposure. The slope divided by the intermittency correction (0.33) and the assumed constant concentration (e.g., 50 $\mu g/m^3$ for 29 years) and multiplied by attained age provides the excess relative hazard to determine the increase of lung cancer rates for the lifetable calculation of the unit risk. The resulting unit risks are presented in Point II in Table 7-3, and closely parallel the results for the case-control study (Point I). The highest values in that set are for the assumption that workers on trains have a ramp (1,50) pattern of exposure. For the ramp pattern the result is a 95% UCL of 1.8×10^{-3} $(\mu g/m^3)^{-1}$ and a MLE of 1.3×10^{-3} $(\mu g/m^3)^{-1}$. For the roof (3,50) pattern of exposure, the procedure is similar, but the exposure scale is increased by the ratio 65/29, representing the ratio of area under the EF of the roof to the area under the EF of the ramp. The result is a 95% UCL of 8.2×10^{-4} $(\mu g/m^3)^{-1}$ and a MLE of 5.7×10^{-4} $(\mu g/m^3)^{-1}$. The lowest values in the set are for the roof (10,50) pattern of exposure. Using a similar approach, multiplying the exposure scale by the AUC ratio of 191/29, the 95% UCL for lifetime unit risk is 2.8×10^{-4} $(\mu g/m^3)^{-1}$, with an MLE of, 1.9×10^{-4} $(\mu g/m^3)^{-1}$.

7.2.4.3.1 DISCUSSION OF RESULTS

The investigation of the forms of the model using Poisson regression explored the use of categorical covariates, calendar year and age-at-start-of-follow-up that verified the categorical trend with exposure that Garshick *et al*. (1988) had obtained for relative hazard by using a Cox regression with calendar year as the principal time scale and age-at-start-of-follow-up as a

covariate. This result was an elevated relative risk (relative hazard) for the middle durations of exposure and an apparent rise at the highest exposure, albeit with large error bars. Crump (1997) found by direct comparison a close correspondence of results for this Poisson regression and a Cox regression that replicated Garshick *et al*.

The investigation also explored the use of a general model with the categorical covariates, calendar year and attained age, that verified the categorical results for relative risk in Crump *et al*. (1991) and Crump (1997). This result showed a rise and then an apparent fall of relative risk for increasing exposure. Age and calendar year are important determinants of lung cancer rate, and Crump (1997) has argued that this choice should be used for covariates because it is the most accurate in characterizing background rates and, further, that a fall of relative risk at the higher exposure, obtained for this choice of covariates, is not consistent with an exposure response.

It should be kept in mind that the categorical trends of the relative risk with duration of exposure are all used to represent a large cloud of observed points of incidence as a function of duration of exposure. See Figure D-7 for an example. Appendix F indicates that the discrepancy between the results of Garshick *et al*. and of Crump *et al*. may be more apparent that real. The slopes for the relative risk are significant for both these choices of covariate, but the slope for the use of calendar year and age-at-start is about twice that for the use of calendar year and attained age. The latter slope is larger, though less significant, than the identical slope obtained in the present analysis using continuous forms of either pair of covariates. The use of the continuous form of the covariates appears to have a salutary effect on reducing the variance of the slope estimate. This choice allows some flexibility, but not a lot, in describing time trends.

7.2.4.4 COMPARISON TO REANALYSES THAT APPLIED TIME-VARYING EXPOSURE CONCENTRATIONS TO THE INDIVIDUAL DATA

Appendix D presents our calculations of slope and unit risk based on exposure since 1945 using different models, different selections of data and different exposure assumptions from those in this chapter. These analyses explore the effect of the different approaches using more accurate models and exposure assumptions than in this chapter. In agreement with Garshick (1991), background concentration is subtracted from all measured concentrations so that the unexposed workers have zero concentration. The resulting unit risks are given in Point III of Table 7-3. These values may be compared with the values obtained by more approximate calculations in Point II. See also Figure 7-3 for a comparison. Comparison with Point II of the table shows that the UCLs and MLEs for unit risk for the general models in Appendix D are less than 25% greater than their approximate counterparts in this chapter. Also the ratio of the value for the ramp (1,50) to that of the roof (3,50) obtained for the more approximate approach is similar to that for general model in Point III. Finally, in Point III the most accurate models are likely to be the multistage models with a late stage sensitive to diesel exhaust exposure. The unit risks from the multistage models are about 3-fold less than the corresponding general model.

In a reanalysis that applied the ramp exposure pattern to the individual data for the Garshick *et al*. cohort, Crump *et al*. (1991) reached three main conclusions, summarized at a Cal/EPA diesel exhaust workshop in 1994: (1) Under-reporting of death became noticeable in the last four years

of follow-up in the study. (2) In analyses that (a) utilized a different model from that reported by Garshick *et al*., (b) but assumed like Garshick *et al*. that exposure started with the start of follow-up and (c) incorporated the full cohort and full follow-up, there was a falling phase of relative risk with categories of increasing cumulative exposure. (3) Slopes determined for cumulative exposure using different groups within the cohort and different measures of exposure concentration and increasing exposure with dieselization of US railroads since 1945 were generally negative, with many being significant statistically.

In this chapter the incomplete follow up of Point (1) is allowed for as in Garshick *et al*. (1988) and Crump *et al*. (1991) and Crump (1997) by controlling for calendar year. Point (2) is one of the points in the discussion just above.

Staff also undertook an investigation of how the significantly negative slopes in Point (3) could have been obtained when the results in Point (2) indicated a considerable overall excess risk with diesel-exhaust exposure, even though there were various degrees of falling phase in the various results. The finding of the investigation was that the result in Point (3) was based on allocating exposure category using insufficiently fine time categories (Dawson, 1995). Subsequent to this finding, Crump (1995) presented corrected calculations of slope, still concluding that there is no effect of diesel exhaust on lung cancer.

In contrast to the present results, the interpretation offered in the recent work of Crump (1995, 1996a, 1996b), who used general models like some of those in Appendix D but did not subtract background concentration, is that there is no convincing evidence of risk. Nevertheless, though none of Crump's results have a statistically significant positive slope, many have a 95% UCL for unit risk that is above zero, ranging up to 1.6×10^{-3} (lifetime-$\mu g/m^3)^{-1}$ for ETS-adjusted RSP, the UARP of Crump (1996a). Using the justification of a need to determine a reasonable numerical estimate of the upper limits on human risk, this value of unit risk may be considered the top of the range determined in Crump (1996a), and it is in good agreement with the top of the range in the present work. This approach to the use of the 95% UCL in the case of nonsignificance is similar to that of Harris (1983). Furthermore, Crump (1996a) presented results showing that for the most plausible assumptions, using all measures of particulate matter which were corrected for the effect of environmental tobacco smoke, and using the simple internally and externally controlled models, and excluding shop workers and the last four years of follow-up, the analyses obtain positive slopes for the maximum likelihood estimate.

Issues discussed concerning the divergence between the finding of no risk and the finding of a risk for the case in which background is not subtracted are described in Appendix E, where the above unpublished references are listed. Appendix F discusses more generally the effect of different assumptions in all the analyses of the cohort to date.

Staff concludes from these analyses that, subject to many uncertainties, including those mentioned immediately below and those pointed out in Crump (1995, 1996a, 1996b), the range of 95% UCL for unit risk for the Garshick *et al*. cohort data is 1×10^{-4} to 2×10^{-3} (lifetime-$\mu g/m^3)^{-1}$.

7.2.5 SOURCES OF UNCERTAINTY IN THE QUANTITATIVE RISK ESTIMATES, BASED ON THE GARSHICK *ET AL*. (1987a, 1988) STUDIES.

The above estimates contain a number of assumptions which could have an effect on the unit risk estimation. These are outlined below.

1. Assumption that the exposure-response is linear at low levels of exposure. The data published in the Garshick *et al*. (1988) cohort and (1987a) case control studies do not allow assessment of departure of hazard trend from linearity at continuous low exposure. The possibility of a threshold at lower doses cannot be excluded in these analyses of the data. Theories of carcinogenesis for a cancer that already has a substantial background, as does lung cancer, suggest that the risk would not generally have a threshold and in the rare case of having a threshold, it would be very difficult to determine. If a threshold does exist above ambient exposure concentrations, then the interpolation of a linear relationship at low exposure tends to overestimate the true risk, which could be negligible at low levels of exposure. Here, the range of extrapolation is relevant.

2. Assumption that mortality rates approximate incidence rates. Because lung cancer usually proves fatal within a few years of diagnosis, this assumption is not a major limitation. Strictly, the potency estimate is for death from lung cancer, rather than lung cancer incidence, which would result in a slightly higher estimate.

3. Assumption that the respiratory particulate fraction is representative of diesel exhaust. Diesel exhaust consists of many hundreds or thousands of separate components. However, for the Garshick *et al*. studies (1987a,1988), information is only available on the respirable particulate fraction. This would not be a problem provided that the components of diesel exhaust were always present in constant proportion to one another. However, it appears that this is not the case (Woskie *et al*., 1988b). Despite this, since the respirable particulate fraction contains many of the carcinogenic components of diesel exhaust adsorbed onto the surfaces of the particulate matter, relying on this fraction as a surrogate for total diesel emissions is not expected to be a serious limitation.

4. Assumption that risk relationships determined from workplace exposures may be extrapolated to environmental exposures. The assumption is that environmental exposures accumulated over a lifetime would yield the same risk as intermittent higher level workplace exposures. Because of a possible threshold, this extrapolation is a source of uncertainty.

However, the range of extrapolation is not large. The statewide average exposure concentration due to diesel particulate is 1.54 $\mu g/m^3$ (Part A). The ratio of the worker's lifetime equivalent exposure to the environmental value is 42 (64 /1.54 = 42).

Any differences between environmental and occupational exposures to diesel exhaust owing to differences in environmental transformation are not accounted for in this analysis.

5. Assumption that the shop workers' exposure was uncertain enough that it was prudent to exclude them from some of the analyses. Many of the shop workers were in shops near the engines sheds, which had very high exposures when diesel engines were running without modern ventilation systems. The exposure concentrations in those conditions are difficult to estimate but might be approximated by using historical nitrogen dioxide measurements in Woskie *et al*. (1988b). The exposures appear to be high enough to suggest the need for a lung burden model to obtain effective exposure, raising the level of difficulty and uncertainty of such an analysis. Other shop workers were in facilities not subject to diesel exposures. In the diesel exposed shops, measurements in 1982-83 by Woskie *et al*. (1988b) for shop workers were about twice the levels of train workers, above background. There does not seem to be any useful information on the proportion of shop workers in the unexposed or lesser exposed shops, thus making it difficult to include the shop workers in the quantitative risk assessment.

Garshick *et al*. (1988) reported that with shop workers excluded, estimates of relative hazard for lung cancer, particularly in the longest exposed group, actually went up a little rather than down, as one would expect after excluding the most exposed group. One possible factor of explanation is random variation. Another is that a large proportion of the shop workers, perhaps more than the unbiased estimate of one half, were apparently not exposed to diesel exhaust or to the measured levels of diesel exhaust. If this proportion were enough more than half, then, whether or not shop workers were included would could give about the same relative hazard. On the other hand, this finding is also consistent with no effect of diesel exhaust on lung cancer.

6. Assumption that the exposure pattern of the train workers had a peak concentration of diesel exhaust exposure in 1959 that was effectively one to ten times the measured concentration at the end of the study and that the concentration on either side of the peak varied linearly with time. This assumption was made for the Garshick *et al*. (1988) cohort and the Garshick *et al*. (1987a) case-control analyses in an attempt to take into account first the documented increase of the use of diesel locomotives from 1945 to 1960 and second the decline of the proportion of smoky locomotives from 1960 on, as newer, cleaner locomotives were purchased. The actual degree of elevation and the shape of the pattern of concentration is subject to considerable uncertainty. The final risk result depends on the value of the peak in a somewhat proportionate manner. This assumption differed from the two assumptions of constant exposure from 1959-1980 in the analysis of McClellan *et al*. (1989). Nevertheless, the exposure concentrations reported are consistent with values used in this analysis.

Crump *et al*. (1991) offered the alternative assumption that exposure increased linearly with time from 1945 to 1959 and was constant throughout the follow-up period, 1959-1980. This "ramp" pattern of exposure seems less plausible than the peaked "roof" pattern that was used because of the reports of smokier engines in the early period. Use of the ramp pattern leads to a prediction of unit risk that is somewhat greater than twice the value obtained for the roof pattern. The ramp pattern is expected to furnish an instructive lower bound on concentration; so the risk calculated probably serves as an upper bound for the effect. The roof pattern is our best reconstruction of exposure pattern: So the difference between the effects calculated for the two provides a useful measure of uncertainty due to the possible patterns of exposure. Appendix D discusses some advantages that the roof pattern has in fitting models to data.

7. Assumption that tobacco smoking did not bias the finding of a positive trend of hazard with exposure to diesel. One of the studies (Garshick 1987a) was adjusted for smoking and thus was not biased by cigarettes smoking. For the cohort study (Garshick 1988), with known smokers constituting up to 80% of the various categories of the cohort (Garshick, 1987b), this possible bias is of concern. Such a bias upwards could occur in two ways: (1) A positive correlation between the duration of diesel exposure and degree of tobacco smoking would tend to bias the hazard estimates upwards. This could occur, for example, (a) if employees in jobs exposed to diesel exhaust smoked more than those unexposed or (b) if the longer the duration of exposure the more the exposed employees smoked. (2) Smoking may cause the trend of relative hazard to rise more substantially with exposure to diesel exhaust than does the trend for non-smokers. This could occur, for example, if smoking tended to interact synergistically with diesel exhaust. Evidence against the situation in Point (1a) is that omitting the unexposed group from several of the analyses still gives a positive exposure-response relationship. Evidence against Point (2) is that the Garshick *et al*. (1987a) case-control study looked for and did not find any interaction between smoking and diesel exhaust exposure and that Hattis and Silver (1992) made theoretical calculations giving ratios of 5 to 6 for lung cancer risk of continuing smokers to non-smokers at inhaled concentrations of 1 to 100 $\mu g/m^3$, essentially independent of concentration in that range. Evidence against all points (1a), (1b) and (2) comes from the Garshick *et al*. (1987a) case-control study. As Section 7.2.1 pointed out, the case-control study drawn just after the end of the cohort study from the same general population of railroad workers, gave the same odds ratio (1.4) for 20 years exposure to diesel exhaust in workers less that 65 years of age, whether adjusted for smoking or not. Thus, even though smoking has a potential for confounding the risk estimates in the present analyses, it appears unlikely that any bias would be substantial. Nevertheless, this consideration is a source of uncertainty in the results of the cohort study.

8. Additional uncertainties in the mathematical aspects of the modeling. (a) Errors in exposure estimates are not explicitly modeled. The correction for this effect is expected to be small. (b) Inter-individual variability in dose-response relations and in lag from carcinogenesis to death is not explicitly modeled. Thus the use of Poisson regression results in overdispersion of the fit. The distributional results could be interesting and useful if the data were extensive enough to support such an analysis. The current results use one simple linear relationship to characterize a gross overall effect. (c) The form of the model and the assumptions about data selection are uncertain. See Appendix F. The use of several different and rather simple models explores some of the variability due to this uncertainty.

9. Assumption that the study populations respond similarly to the general population. The study populations were healthy male workers. Therefore these studies do not provide information bearing on the possible greater or lesser sensitivity of other groups such as women and children or those with impaired health. Since it is not possible to quantify the risk based on exposure of women, children or other possible more susceptible members of the population, OEHHA uses the 95% upper confidence limit on the slope of the dose-response curve in male workers.

10. Control for healthy work effect. Workers are frequently observed to have lower rates of morbidity and mortality in epidemiological studies than the general population. This finding is

referred to as the "healthy worker effect". The analyses here of the Garshick *et al*. data (1987a, 1988) control for the healthy worker effect by internal standardization, using unexposed members of the cohort as a reference population.

7.2.6 RELATIONSHIPS BETWEEN THE TWO STUDIES USED

The Garshick *et al*. (1987a) case-control study and the Garshick *et al*. (1988) cohort study drew on the population of U. S. railroad workers at about the same time. The case control study obtained data on the prior history of lung cancer cases occurring in 1983 and matched controls with other causes of death. The cohort study collected data on the health and work status of members from 1959 through 1980 and also collected data on the prior history of all members. While there could be no deaths in common in the two studies, some survivors of the cohort study could have died in 1981, the year of the case-control study. The case-control study obtained death certificates for about 13,000 total deaths in 1981 while the cohort study obtained up to about 1300 death certificates in each of the few years previous to and including 1978, the last year without a major decline of apparent reporting of deaths. Very different inclusion criteria were used for the two studies. Assuming that the actual yearly number of deaths continued to stay about constant up to 1983, the chance of a person in the case-control (cc) study being in the cohort (co) is the same as the ratio of cohort deaths in 1982 to total deaths in 1982. Thus,

$$P(co \mid cc) = P(co\ death\ 82 \mid death\ 82) = 1{,}300/15{,}059 = 0.086.$$

The chance a person in the cohort study being in the case-control study can be determined by using Bayes' Rule with 1) the ratio of case-control deaths in 1982 to all deaths in 1982, 3,641/15,059 = 0.24; and, 2) the ratio of case-control deaths in 1982 to the total numbers in the cohort, 1,300/55,407 = 0.024. Thus,

$$P(cc \mid co) = P(cc \mid co,\ co\ death\ 82) \times P(co\ death\ 82) = 0.24 \times 0.024 = 0.0058$$

Thus, the two studies can be considered nearly independent samples of that railroad worker population.

The case-control study had smaller numbers and presented less age-specific information. Nevertheless this study included the important feature of an evaluation of the effect of smoking among the workers, which the cohort study does not do. The analysis in the case-control study showed that the odds ratio was the same whether adjusted for smoking status or not, even though more cases than controls were cigarette smokers and had a greater pack-year history of smoking. This finding is evidence that smoking is unlikely to have been a problem in confounding the cohort study as well. A study of asbestos exposure in railroad workers reported that smoking prevalence was about 80% in older workers, whether with or without asbestos exposure (Garshick *et al*., 1987b).

The UCLs of the linear prediction obtained using the results of the Garshick *et al*. (1987a) case-control study are only slightly above the respective results for the ramp and roof pattern obtained

using the results of the Garshick *et al*. (1988) cohort study for general multiplicative models in Appendix D. See Table 7-3 and Figure 7-3.

7.2.7 COMPARISON TO RESULTS OF OTHER STUDIES

Harris (1983) analyzed data from the London Transport Worker Study to obtain another estimate of unit risk. Comparison of the cancer death rates for the high exposure group with those for London males gave observed-to-expected ratios that were less than 1, leading the original authors not to make a positive finding. On the assumption that these low values were due to the "healthy worker effect", Harris merged the exposed groups and compared them to the group formed by merging two groups of unexposed individuals. The analysis found that whether or not the original data gave a positive result depended upon the assumptions made about smoking rates and that the best fit assumption did not give a statistically significant effect. Nevertheless, the analysis obtained a 95% UCL of 5×10^{-4} $(\mu g/m^3 \times yr)^{-1}$ for the rate of increase of relative risk of diesel exposure. Applying this slope in the modified California life table gives a unit risk of 1.4×10^{-3} $(\text{lifetime-}\mu g/m^3)^{-1}$. This value of 95% UCL is close to the values obtained in the above assessments for the ramp exposure pattern in the Garshick *et al*. (1988) cohort and (1987a) case-control study.

The results of quantitative risk estimates based on epidemiological data are summarized in Table 7-5. The US EPA 1998 draft *Health Effects of Diesel Exhaust* presents a unit risk based on McClellan *et al.* (1989) of 2×10^{-3} as the top end of their range. Smith (1998) estimated a unit risk of 3×10^{-4} based on the smoking-adjusted pooled relative risks in the OEHHA meta-analysis. Steenland (1998) provides a range of 0.1 to 1.6×10^{-3} based on his analysis of the relationship between cumulative exposure and lung cancer risk in diesel exhaust-exposed truck drivers. OEHHA have also included in this table bracketed risks and the 90^{th} % confidence interval of the bracket as described in Section 7.2 based on the meta-analysis in Appendix C.

7.3 CONCLUSION

Based on the human data, the principal finding of this quantitative risk assessment is a range of lifetime unit risk (95% UCL) as shown in the right-hand column of Table 7-3. The lowest value in the range is 1.3×10^{-4}, and the highest value is 2.4×10^{-3}. The geometric mean unit risk obtained from these end points of the range of values is 6×10^{-4} $(\text{lifetime-}\mu g/m^3)^{-1}$. The geometric mean provides information on the central tendency of the range and is not to be confused with a best estimate identified from the available calculations. The lower end of the range is the rounded value for both forms of multistage model using the roof exposure pattern for the data of the Garshick *et al*. (1988) cohort study of U.S. railroad workers. OEHHA concludes that incorporation of the roof exposure pattern and biologically-based analyses improve the unit risk estimates. Consequently, unit risk values incorporating this information, those at the lower end of the range, provide more scientifically defensible values. The upper end of the range is obtained using the published results of the Garshick *et al*. (1987a) case-control study for US railroad workers. Figure 7-3 and Table 7-3 show the overlapping values obtained for up to five different patterns of exposure in each of these two studies and for two different models, a general

model and a multistage model in the cohort study. Figure 7-3 also displays diagramatically the current 95% UCLs for unit risks in comparison to values obtained by others.

Duration analyses adapted to five different chronological reconstructions of patterns of exposure calculate overlapping risks for the two studies of railroad workers. These analyses provide slopes of risk with increasing cumulative exposure. For the case-control study, based on the slope from the logistic regression for the study the 95% UCL obtained for unit risk ranges from 3.6×10^{-4} to 2.4×10^{-3}. (lifetime $\mu g/m^3)^{-1}$. For the cohort study based on a new reanalysis of individual data, the 95% UCL obtained for unit risk ranges from 2.8×10^{-4} to 1.8×10^{-3} (lifetime $\mu g/m^3)^{-1}$. The top of these ranges is consistent with the result obtained by Harris (1983), 1.4×10^{-3} (lifetime-$\mu g/m^3)^{-1}$, for London transport workers (see Table 7-5).

As indicated in Section 6.2 and in the meta-analysis in Appendix C, and in the conclusion of Cohen and Higgins (1995), there is a consistent small increase of relative lung cancer risk above 1 in a number of occupational studies associated with diesel exhaust exposure. The task of the quantitative risk assessment in this chapter and Appendix D is to provide reasonable estimates of the human risk.

The linear relationships of risk to exposure determined from the individual data that were analyzed in the Garshick *et al*. (1988) railroad worker cohort study for quantitative risk assessment has led to two divergent interpretations. The differences in approach and interpretation are summarized in Appendix E for calculations that do not subtract background. Crump's reanalysis (1995, 1996a, 1996b) essentially finds no statistically significant increase of risk with diesel-exhaust exposure. Appendix F discusses how the various assumptions taken in calculations using the individual data affect the resulting risks. The work of Dr. Crump suggests that the results are not completely robust to the mode of analysis. Nevertheless, as discussed in Section 7.2.4.4, for the most plausible assumptions in his analyses Crump (1996a) presents UCLs for unit risk well within the human-based range calculated in this document and maximum likelihood estimates of slope that are all above zero. The present results in this chapter and in Appendix D find statistically significant increases with a range of 95% UCL for unit risk of 1.3×10^{-4} to 1.9×10^{-3} (lifetime-$\mu g/m^3)^{-1}$.

We present estimates of cancer risk based on data in rats in Appendix G. On balance, the human data lend more confidence in the prediction of human risks than the data from the rat studies because of the uncertainties of extrapolating from rats to humans, especially in the context of a substantial particle effect. These uncertainties in this species extrapolation appear to outweigh the uncertainties of using the epidemiological results -- uncertainties of the actual exposure history, the modeling and data selection. The uncertainty in the extrapolation from animal data is difficult to quantify, but is likely to be much greater than in using human data.

The strengths and weaknesses of calculating population risks using the human studies (Garshick *et al*. 1988, Garshick *et al*. 1987a) and the animal bioassays (Mauderly *et al*. 1987a, Brightwell *et al*. 1989, Heinrich *et al*. 1995, Ishinishi *et al*. 1986a, Nikula *et al*. 1995) are presented in Table 7-6. This summary is based on the issues discussed above, especially in Section G.2.8 and

Section 7.2. As discussed above (Section G.4.3), an approximate correction for smoking would raise the rat-based unit risks into near coincidence with the human-based unit risks as applied to the California population.

The range of lifetime unit risk (95% UCL) per $\mu g/m^3$ is provided in the right-hand column of Table 7-3. The Air Resources Board has estimated that the average annual ambient concentration of diesel exhaust to which Californians are exposed is 1.54 $\mu g/m^3$, including both indoor and outdoor exposure. The upper limit of potential additional cancer cases over a lifetime in California can be estimated using the cancer unit risk values in Table 7-3 and the Air Resources Board estimate of average ambient concentration of diesel exhaust to which Californians are exposed. This estimate is a range from 200 to 3600 additional cancer cases for every one million Californians over a 70 year lifetime. The estimate was derived by multiplying the average statewide concentration of diesel exhaust (1.54 $\mu g/m^3$) times the highest and lowest 95% UCL values of cancer unit risk found in Table 7-3. The estimates were rounded to one significant digit to avoid the implication of undue precision in the values. OEHHA concludes, based on analyses presented in Chapter 7 and Appendix D, that the more scientifically valid unit risk values and subsequent estimates of the upper limit of potential additional cancer cases are near the lower end of the ranges.

Table 7-1. MODIFIED LIFE TABLE TO ESTIMATE CALIFORNIA LUNG CANCER RISK BY AGE CATEGORY

Uses: (1) California Department of Finance population projection for July 1, 1991;
(2) deaths by age... Table 5-6, Vital Statistics of California, DHS 1991;
(3) five-year cancer incidence, Table XIV -5, Cancer Incidence and Mortality in California by Detailed Race/Ethnicity, 1988-1992, DHS 1995
(4) includes effect of slope calculated for Garshick et al. case control study, assuming concentration of 50 $\mu g/m^3$

#	p(i)= 1-q(i)= exp(-5*m(i))	C(i)= C(i-1)*p(i-1)	l(i)	l(i)*[1 + slope/0.33 /50*(i+2)]* 1100/1450	pl(i) l(i)/m(i)*q(i)	u(i)= pl(i)*C(i)	S(i)= S(i-1)+u(i-1)		REFERENCE DATA n(i)	d(i)
period	P (survival to age i + 4, given survival to age I)	cumulative P (survival to age I)	California 1988-92 annual lung cancer death rate per [10^5] in (i, i+4)	Annual lung cancer death rate increased by relative hazard. per [10^5] in (i, i+4)	P (lung cancer death, given survival to age I)	unconditional P (lung cancer death in (i, i+4))	cumulative P (lung cancer death by age i+4)		California 1991 population projection	California 1991 all deaths
						0				
1	0.990	1.000	0.000	0	0.00E+01	0.00E+01	0.000000		2622793	5468
2	0.999	0.990	0.000	0	0.00E+01	0.00E+01	0.000000		2272842	450
3	0.999	0.989	0.000	0	0.00E+01	0.00E+01	0.000000		2072016	520
4	0.995	0.987	0.000	0	0.00E+01	0.00E+01	0.000000		2031176	1943
5	0.995	0.983	0.100	0.102470156	5.11E-05	5.02E-05	0.000005		2542302	2769
6	0.994	0.977	0.200	0.206063111	1.03E-04	1.00E-04	0.000015		2829444	3282
7	0.992	0.972	0.700	0.725150682	3.61E-04	3.51E-04	0.000050		2922466	4444
8	0.990	0.964	2.600	2.708013198	1.35E-03	1.30E-03	0.000180		2587937	5328
9	0.987	0.954	8.200	8.586691739	4.27E-03	4.07E-03	0.000587		2282160	5989
10	0.982	0.942	25.000	26.31928802	1.30E-02	1.23E-02	0.001816		1667721	6122
11	0.974	0.925	56.300	59.58710434	2.94E-02	2.72E-02	0.004535		1337454	7129
12	0.959	0.901	103.000	109.5919489	5.37E-02	4.83E-02	0.009368		1140360	9547
13	0.938	0.864	167.000	178.6254537	8.65E-02	7.47E-02	0.016839		1110088	14249
14	0.908	0.810	231.000	248.3775495	1.18E-01	9.59E-02	0.026430	(target)	1062044	20388
15	0.864	0.736	295.000	318.8482362	1.48E-01	1.09E-01	0.037343		841694	24643

Table 7-2 Number of Workers in the Exposure Categories and the Cohort Averages of the Worker Exposure Concentration Following the Garshick *et al.* (1988) Cohort Study.

Exposure status	Career group	Number of workers	Subsequent exposure concentration[a] ($\mu g/m^3$)
Uncertain	Shopworkers	12,092	141 (those exposed)
Exposed[b]	Engineers, firemen	11,005	71
	Brakemen, conductors, hostlers	18,285	89
Unexposed[c]	Clerks	10,475	33
	Signalmen	3548	58

[a] Exposures reported by Woskie *et al* (1988b) for these career groups, based on measurements of ETS-adjusted RSP, circa 1982-3.

[b] For all exposed workers in the table, except for those shopworkers who were exposed, the temporal exposure patterns are assumed to be the same, and the concentrations are close to each other; so a simple population-weighted average for the two career groups characterizes the average concentration for the exposed group, train workers, circa 1982-83:

$$(11{,}005 \times 71 + 18285 \times 89) / (11005 + 18285) = 82\ \mu g/m^3$$

[c] For all unexposed workers (background) in the table except for those shopworkers who were unexposed, the concentrations are close to each other; so a simple population-weighted average for the two groups characterizes the average background concentration, circa 1982-83:

$$(10475 \times 33 + 3548 \times 58) / (10475 + 3548) = 39\ \mu g/m^3.$$

Table 7-3 Values from Unit Risk for Diesel Exhaust from Using Hazard Slope on Exposure Measure in California Life-Table.
Garshick *et al*. (1987a, 1988) Studies of U.S. Railroad Workers.

	$q1\ (\mu g/m^3)^{-1}$	
	MLE	95% UCL
I. Case-Control study (1987a) using published slope coefficient for hazard on years of exposure to diesel exhaust (Section 7.3.3)		
A. Adapted to ramp (1,50) pattern of exposure	1.4×10^{-3}	2.4×10^{-3}
B. Adapted to roof (2,40) pattern of exposure	1.1×10^{-3}	1.8×10^{-3}
C. Adapted to roof (3,50) pattern of exposure	6.2×10^{-4}	1.0×10^{-3}
D. Adapted to roof (3,80) pattern of exposure	3.9×10^{-4}	6.6×10^{-4}
E. Adapted to roof (10,50) pattern of exposure	2.1×10^{-4}	3.6×10^{-4}
II. Cohort study (1988) using individual data to obtain a slope for hazard on years of exposure to diesel exhaust (Section 7.3.4) Continuous covariates: (attained age and calendar year) or (age-at-start-of study and calendar year)		
A. Adapted to ramp (1,50) pattern of exposure	1.3×10^{-3}	1.8×10^{-3}
B. Adapted to roof (2,40) pattern of exposure	9.9×10^{-4}	1.4×10^{-3}
B. Adapted to roof (3,50) pattern of exposure	5.7×10^{-4}	8.2×10^{-4}
D. Adapted to roof (3,80) pattern of exposure	3.6×10^{-4}	5.1×10^{-4}
E. Adapted to roof (10,50) pattern of exposure	1.9×10^{-4}	2.8×10^{-4}
III. Cohort study (1988) applying time varying concentrations to individual data to obtain a slope of hazard on exposure (from Appendix D)		
A. Ramp (1,50) pattern of exposure		
1. General multiplicative model with age-at-start-of-study and U.S. rates as categorical covariates	1.2×10^{-3}	1.9×10^{-3}
2. 6th/7-stage model with age-at-start-of study as categorical covariate	2.4×10^{-4}	3.8×10^{-4}
B. Roof (3,50) pattern of exposure		
1. general multiplicative model with age-at-start-of-study and U.S. rates as categorical covariates	5.1×10^{-4}	7.2×10^{-4}
2. 6th/7-stage model with age-at-start-of-study as categorical covariate	8.1×10^{-5}	1.3×10^{-4}
3. 7th/7-stage model with age-at-start-of-study as categorical covariate	1.0×10^{-4}	1.5×10^{-4}

Table 7-4 Conversion of California Population Lifetime Risk to Non-smoker Lifetime Risk.

Annual incidence rate function for lung cancer (Doll, 1971): $= i_{50} (x/50)^k$,

where

i_{50} = annual incidence at age 50 for log-log linear fit,

x = age in years,

k = exponent for Armitage-Doll model.

For American non-smoker data:

$i_{50} = 6.9 \times 10^{-5}\ yr^{-1}$,

$k = 4.0$

scaled from a plot in Doll (1971).

The risk, using this incidence rate function for the 70-year target lifetime, =

$$\int_0^{70} i_{50} (x/50)^k \, dx = 50\, i_{50} \left[(x/50)^{k+1}/(k+1)\right]_0^{70} = 0.0037.$$

Ratio of the California lifetime risk (from Table E-1) to this non-smoker lifetime risk =

$$0.25 / 0.0037 = \underline{6.8}\ .$$

Table 7-5. Comparison of Other Organizations' Estimated 95% Upper Confidence Limits of Lifetime Risk per μg/m^3 Diesel Particulate Matter from Risk Assessments Based on Epidemiologic Data with OEHHA Estimates

Method	Unit Risk/Range	Basis of Assessment	Reference
Epidemiologic analysis[a]	**1.3 to 7.2 x 10^{-4}**	**cohort study, time varying conc., roof (3,50) pattern**	**OEHHA, Part B, Appendix D**
Epidemiologic analysis	**2.8 x 10^{-4} to 1.8 x 10^{-3}**	**cohort study of Garshick *et al*., 1988**	**OEHHA, Part B, Section 7.3.4**
Epidemiologic analysis	3 x 10^{-4}	based on smoking-adjusted pooled RR	Smith, 1998
Epidemiologic analysis	**3.6 x 10^{-4} to 2.4 x 10^{-3}**	**case-control study of Garshick *et al*., 1987**	**OEHHA, Part B, Section 7.3.3**
Epidemiologic analysis	**3.8 x 10^{-4} to 1.9 x 10^{-3}**	**cohort study, time varying conc., ramp (1,50) pattern**	**OEHHA, Part B, Appendix D**
Epidemiologic analysis	0.1 to 1.6 x 10^{-3}	case-control study of U.S. truck drivers based on cumulative exposures	Steenland, 1998; Federal Register Notice, Volume 63, Number 68, Thursday, April 9, 1998. 30 CFR, Parts 72 and 75. Page 17542[b]
Epidemiologic analysis	1.4 x 10^{-3}	London transport study[c]	Harris, 1983
Epidemiologic analysis	2 x 10^{-3}	epidemiologic data of Garshick (top end of U.S. EPA's range)	U.S. EPA, 1998
Epidemiologic analysis	1.3 x 10^{-4} to 1.3 x 10^{-2} 90% CI = 1.6 x 10^{-4} to 1.2 x 10^{-3}	using smoking adjusted RR and exposures of 5 or 500 μg/m^3	OEHHA, Part B, Section 7.3; bracketed risk bounds

a - Bolded values are included in OEHHA's range of risk.

b - Range is based on a working lifetime of exposure to diesel particulate matter at 500 μg/m³ which yielded estimates of excess lung cancer risk ranging from about 50 to 800 per 1000 workers based on epidemiological assessment.

c - Obtained by applying Harris' slope of 5 x 10^{-4} (μg/m³ x yr)$^{-1}$ to California life table.

Table 7-6 Human and Animal Information for Quantitative Estimates of Risk.

Information/Advantage[a]	Animal[b]	Human[c]
Accuracy of exposure estimate in study A++	Numerically precise for rats exposed to automobile exhaust	Uncertain for the railroad workers
Ratio of study exposure to human environmental exposure H++	300	7
Similarity of study exposure to present day exhaust A+	Some uncertainty	Some uncertainty. Uncertain quantitative control for smoking and other pollutants
Model to predict risks at human environmental levels H+	Uncertainty of biological responses such as cell proliferation	Some uncertainty of biological responses such as cell proliferation
Applicability to the human process H++	Much uncertainty in pharmacokinetics and pharmacodynamics	No uncertainty
Consistency of results 0	Consistent with other rat results	Consistent with other human results
Accounting for heterogeneity of human population H+	Uncertainty in ability of the rat model to protect sensitive humans	The railroad study considered only white male workers, who may not be most sensitive
OVERALL CONCLUSION H+	Data quality is strong, but applicability to humans at environmental concentrations is uncertain	Exposure data are weak, but unlikely to greatly overstate or understate risks

[a] Symbols: H for human, A for animal, 0 for neither has the advantage. + and ++ represent the strength of the advantage.

[b] Mauderly *et al*. (1987a), Brightwell *et al*. (1989), Heinrich *et al*. (1995), Ishinishi *et al*. (1986a), Nikula *et al*. (1995)

[c] Garshick *et al*. (1988), Garshick *et al*. (1987a)

Figure 7-1 GARSHICK ET AL. (1988) RAILROAD WORKER COHORT: PEAK PATTERN FOR EXPOSURE FACTOR AND THE RESULTING AREA UNDER THE CURVE (AUC)

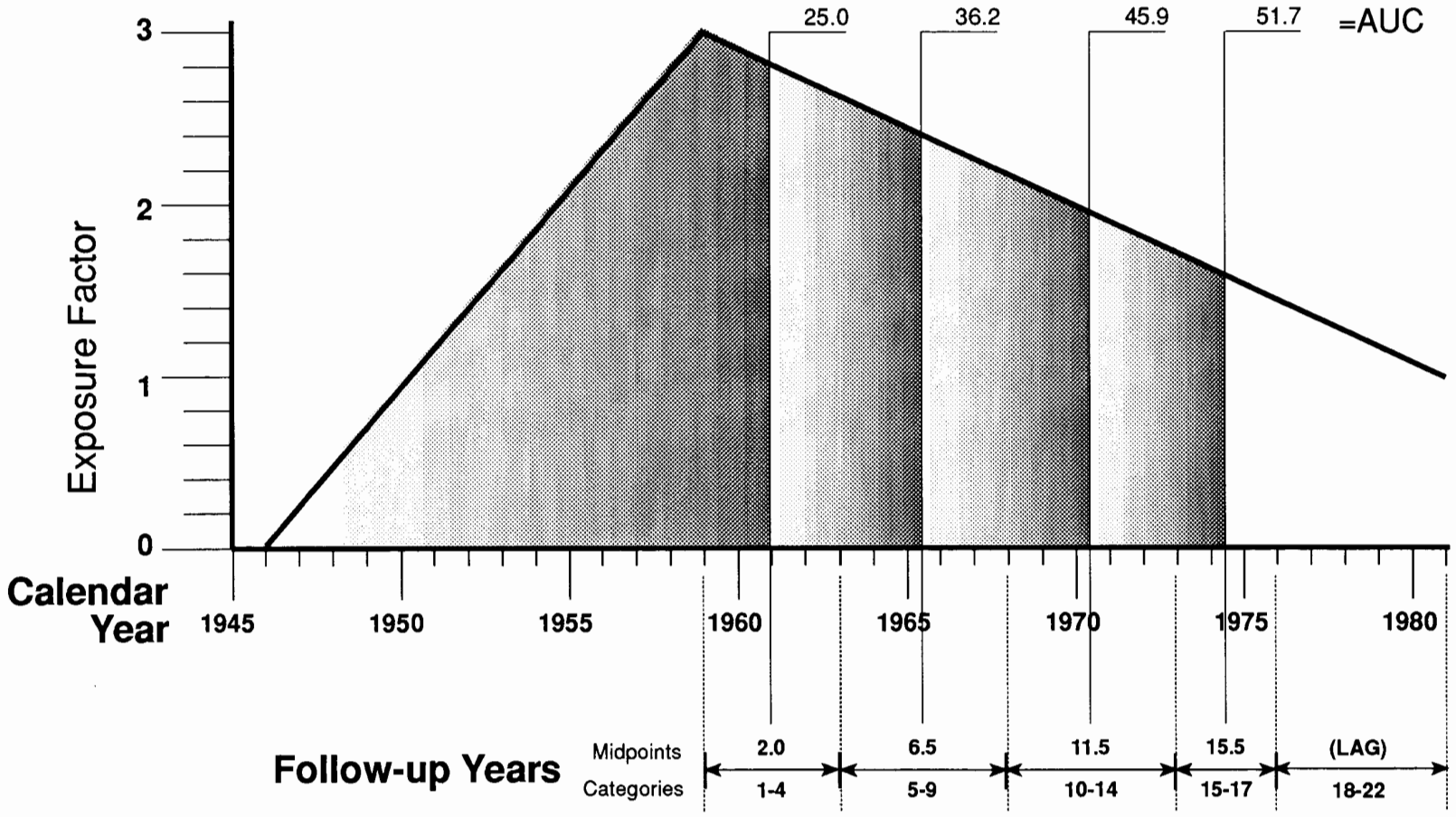


Figure 7-2. Excess Relative Hazard for Duration Analysis from 1952 to Follow-up Year. Attained Age and Calendar Year are Linear and Quadratic Continuous Covariates.

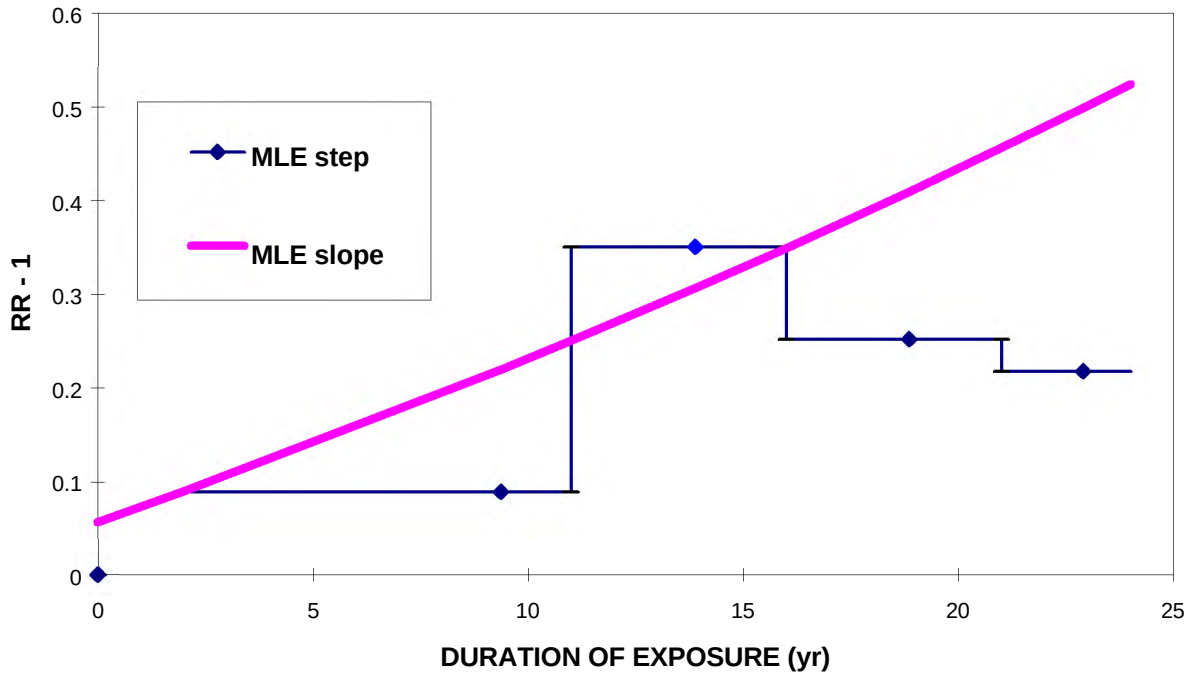


Figure 7-3 95% UCL FOR LIFETIME UNIT RISK FOR HUMANS USING US RAILROAD WORKER STUDIES

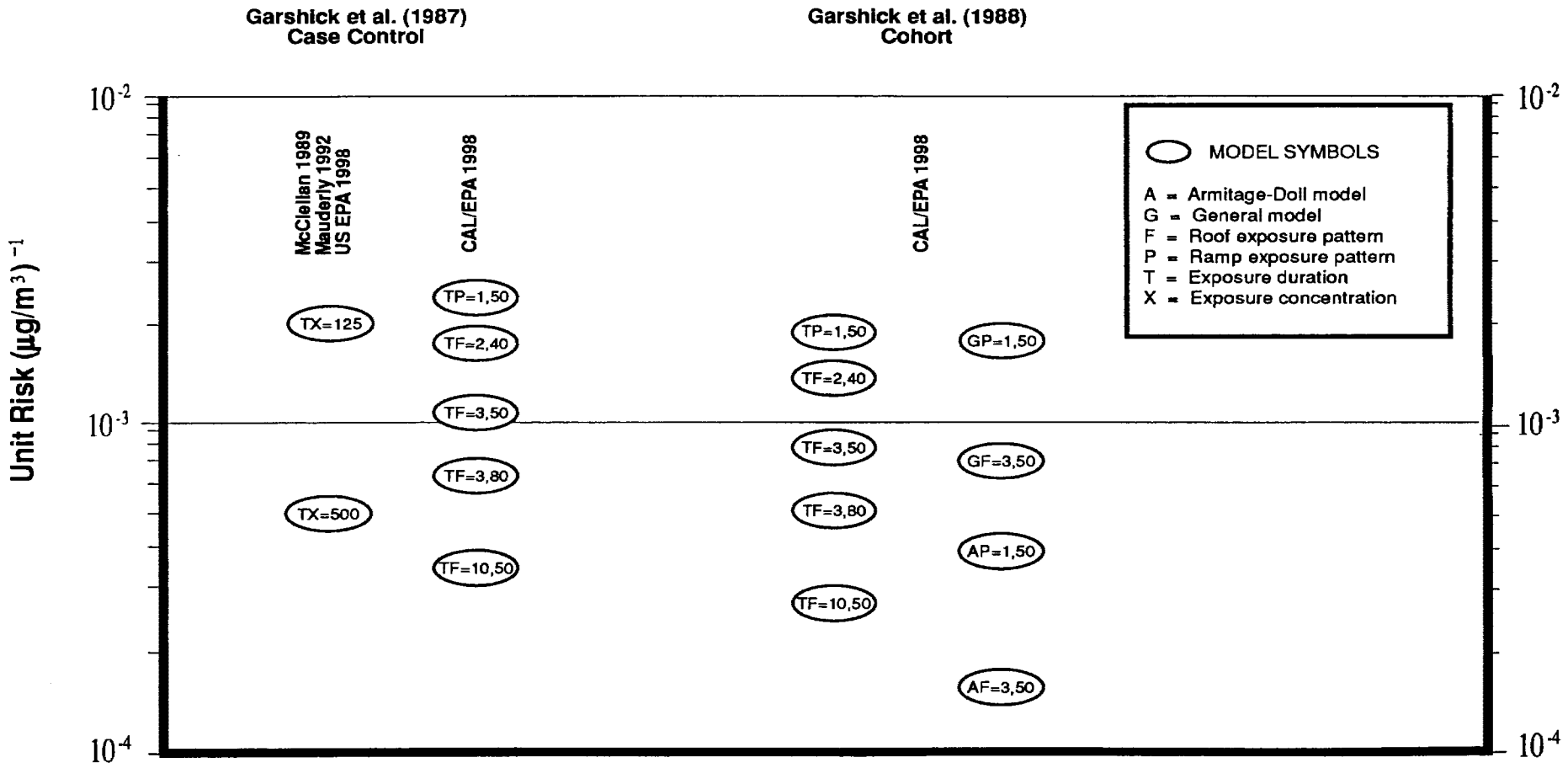
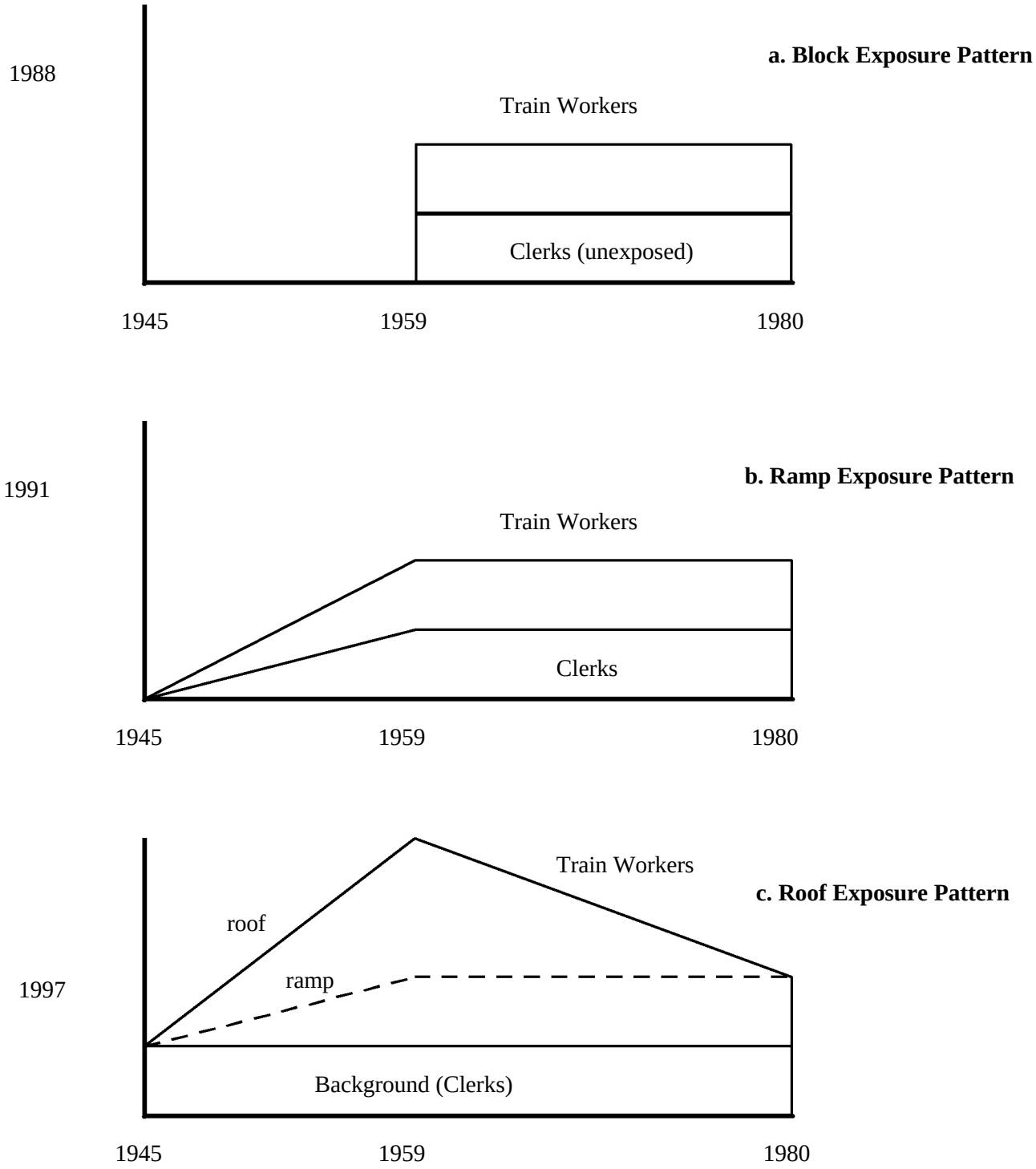


Figure 7-4 Different Exposure Patterns



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APPENDIX A

DIESEL ENGINE EMISSIONS IRIS, U.S.EPA 07/93 (DOCUMENTATION OF THE U.S.EPA RfC) Obtained from IRIS October 22, 1996

REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Substance Name: Diesel engine emissions
CASRN: Not found

The inhalation Reference Concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as carcinogenicity. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is appropriately expressed in units of mg/m^3. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs are derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) developed by U.S. EPA scientists and peer-reviewed. For more information on the interim nature of these methods and future plans see the IRIS News field. RfCs can also be derived for the noncarcinogenic health effects of compounds which are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in the Carcinogenicity Assessment Section of this file when a review of that evaluation is completed.

RfC ASSESSMENT SUMMARY TABLE

Critical Dose: 0.155 mg/m^3 [Study 1 NOAEL(HEC)]
UF: 30 MF: 1 RfC: 5E-3 mg/m^3 Confidence: High
Critical Effect: (1) Histological changes in the lung
(2) Inflammatory, histological and biochemical changes in the lung and impaired particle clearance

	NOAEL (Study 1)	LOAEL (Study 1)
Reported	0.46 mg/m^3	0.96 mg/m^3
ADJ	mg/m^3	mg/m^3
HEC	0.155 mg/m^3	0.30 mg/m^3
Scenario	particle, respiratory	particle, respiratory
Study Type	Chronic Rat Inhalation Study	Chronic Rat Inhalation Study
Reference	Ishinishi *et al.*, 1988	Ishinishi *et al.*, 1988

Other Refs: (2) Mauderly *et al.*, 1988

1) Ishinishi *et al.*, 1988 Chronic Rat Inhalation Study

Critical Effect: Histological changes in the lung

Defined Concentrations:		
	NOAEL	0.46 mg/m^3
	NOAEL(ADJ)	
	NOAEL(HEC)	0.155 mg/m^3
	LOAEL	0.96 mg/m^3
	LOAEL(ADJ)	
	LOAEL(HEC)	0.30 mg/m^3

Scenario: Particle, respiratory effect

Conversion Factors: Human equivalent concentrations were extrapolated from the experimental conditions using the particle retention model of Yu and Yoon (1990) to an assumed continuous human exposure using mass of diesel particle carbon core per unit of surface area in the pulmonary region as the dose metric.

2) Mauderly *et al.*, 1988 Chronic Rat Inhalation Study

Critical Effect: Inflammatory, histological and biochemical changes in the lung and impaired particle clearance

Defined Concentrations:		
	NOAEL	0.353 mg/m^3
	NOAEL (ADJ)	
	NOAEL (HEC	0.042 mg/m^3
	LOAEL	3.47 mg/m^3
	LOAEL (ADJ)	
	LOAEL (HEC)	0.36 mg/m^3

Scenario: Particle, respiratory effect

Conversion Factors:	Human equivalent concentrations were estimated for the experimental conditions using the particle retention model of Yu and Yoon (1990) assuming a continuous human exposure and using mass of diesel particle carbon core per unit of surface area in the pulmonary region as the dose expression.

DISCUSSION OF PRINCIPAL AND SUPPORTING STUDIES

Ishinishi, N., N. Kuwabara, Y. Takaki *et al.* 1988. Long term inhalation experiments on diesel exhaust. In: Diesel Exhaust and Health Risk. Results of the HERP Studies. Entire Text of Discussion. Research Committee for HERP Studies. Japan Automobile Research Institute, Inc. Tsukuba, Ibaraki 305, Japan.

Mauderly, J.L., R.K. Jones, R.F. Henderson *et al.* 1988. Relationship of lung structural and functional changes to accumulation of diesel exhaust particles. Ann. Occup. Hyg. 32: 659-669.

A total of 10 different long-term (>1 year) animal inhalation studies of diesel engine emissions have been conducted. The focus of these studies has been on the respiratory tract effects in the pulmonary region. Effects in the upper respiratory tract and in other organs were not found consistently in chronic animal exposures. The critical studies cited above are derived from research programs on the toxicology of diesel emissions that consisted of large-scale chronic exposures, with exposed animals being designated for the study of various endpoints and at various time points. Each research program is represented by multiple published accounts of results from various aspects of the overall research program, including exposure system, exposure characterization, and various toxicological endpoints. In addition to the critical study, other publications are discussed in the following text for each program, which cumulatively describe the results for the particular exposure conditions. The respiratory system response has been well characterized in terms of histopathology, biochemistry, cytology, pulmonary function, and respiratory tract clearance. The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the phagocytosis of diesel particles by alveolar macrophages. These activated macrophages release chemotactic factors that attract neutrophils and additional alveolar macrophages. As the lung burden of diesel particles increases, there is an aggregation of particle-laden alveolar macrophages in alveoli adjacent to terminal bronchioles, there are increases in the number of Type II cells lining particle-laden alveoli, and particles are present within alveolar and peribronchial interstitial tissues and associated lymph nodes. The neutrophils and macrophages release mediators of inflammation and oxygen radicals. Particle-laden macrophages are functionally altered, resulting in decreased viability and impaired phagocytosis and clearance of particles. The latter series of events may result in the presence of pulmonary inflammatory, fibrotic, or emphysematous lesions.

Histopathological effects of diesel exhaust on the lungs of rats have been investigated by the Health Effects Research Program on Diesel Exhaust (HERP) in Japan (Ishinishi *et al.*, 1986, 1988). In this study, both light-duty (LD, 1.8 L) and heavy-duty (HD, 11 L) diesel engines were operated under constant velocity and load conditions. The exhaust was diluted to achieve target concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD only) mg/m^3 particulate matter. Particle concentrations were determined by filter samples. Actual concentrations were 0.11, 0.41, 1.18, and 2.32 mg/m^3 for the LD engine and 0.46, 0.96, 1.84, and 3.72 mg/m^3 for the HD engine. The number of samples and frequency of sampling is not clear from the published reports. Fischer 344 rats (120 male and 95 female/exposure level for each engine type) were exposed for 16 hours/day, 6 days/week for 30 months. Duration-adjusted concentrations are 0.063, 0.23, 0.67, and 1.3 mg/m^3 for the LD engine and 0.26, 0.55, 1.05, and 2.13 mg/m^3 for the HD engine. Particle size distributions were determined using an Andersen cascade impactor and an electrical aerosol analyzer. The number and timing of the samples are not clear from the published reports, nor is it clear which method was used for the reported results. At the 24-month sampling, the MMD and distribution (sigma g) were 0.22 (2.93) and 0.19 (2.71) μm for the LD engine groups at 2.32 and 1.18 mg/m^3, respectively, and 0.27 (3.18) and 0.22 (2.93) μm for the HD engine groups at 3.72 and 1.84 mg/m^3, respectively (Ishinishi *et al.*, 1988). Particle size data were not reported for the other exposure groups. Hematology, clinical chemistry, urinalysis, and light and electron microscopic examinations were performed. The body weight of female rats exposed to 4 mg/m^3 was 15-20% less than controls throughout the study. A dose-dependent decrease in body weight of the other groups is mentioned, but neither data nor statistical analysis are reported. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 particulate matter or less. At concentrations above 0.4 mg/m^3 particulate matter, accumulation of particle-laden macrophages was observed. In areas of macrophage accumulation, there was bronchiolization of the alveolar ducts, with bronchiolar epithelium replacing alveolar epithelium. Proliferation of bronchiolar epithelium and Type II cells was observed. In these areas, edematous thickening and fibrosis of the alveolar septum were seen. Fibrosis of the alveolar septum developed into small fibrotic lesions. These lesions are collectively referred to as hyperplastic lesions by the authors, and their incidence is reported. From a total of 123-125 animals examined (approximately equal numbers of males and females), hyperplastic lesions were reported in 4, 4, 6, 12, and 87 animals in the LD engine groups exposed to 0, 0.11, 0.41, 1.18, and 2.32 mg/m^3, respectively, and in 1, 3, 7, 14, and 25 animals in the HD engine groups exposed to 0, 0.46, 0.96, 1.84, and 3.72 mg/m^3, respectively. Statistical analysis of these results was not reported, but it appears that there was no difference in the degree of changes in pulmonary pathology at similar exposure concentrations between the LD and the HD series. This study identifies LOAELs for chronically exposed rats at 1.18 and 0.96 mg/m^3 (actual exposure) for LD and HD series and NOAELs at 0.41 and 0.46 mg/m^3 (actual) for LD and HD series. Duration-adjusted concentrations for the LD and HD series are 0.67 and 0.55 mg/m^3 (LOAELs) and 0.23 and 0.26 mg/m^3 (NOAELs). Because the particle sizes are not reported for the NOAEL for the 0.4-mg/m^3 LD group or the 0.5-mg/m^3 HD group, the size distributions for these are assumed to be the same as the next highest group. Thus, for both LD and HD series, the LOAEL(HEC) and NOAEL(HEC) are estimated using the particle deposition and retention model developed by Yu and Yoon (1990) using the same MMAD and sigma g. The resulting LOAEL(HEC) for the LD and HD series are 0.359 and 0.303 mg/m^3, respectively.

The NOAEL(HEC) for the LD and HD series based on the retention model are 0.139 and 0.155 mg/m^3, respectively.

Rats and mice were exposed to target diesel particulate concentrations of 0, 0.35, 3.5, or 7 mg/m^3 for 7 hours/day, 5 days/week for up to 30 months (rats) or 24 months (mice) (Mauderly *et al.*, 1988) in studies performed at the Inhalation Toxicology Research Institute (ITRI). A total of 364-367 rats/exposure level (approximately equal numbers of males and females) were exposed and used for various studies examining different endpoints, including carcinogenicity, respiratory tract histopathology and morphometric analysis, particle clearance, lung burden of diesel particulate matter, pulmonary function testing, lung biochemistry, lung lavage biochemistry and cytology, immune function, and lung cell labeling index. Subsets of animals were examined at 6, 12, 18, and 24 months of exposure, and surviving rats were examined at 30 months. Diesel emissions from a 5.7 L engine operated on a Federal Test Procedure urban driving cycle were diluted and fed into the exposure chambers. Particle concentrations were measured daily using a filter sample, and weekly grab samples were taken for measurement of gaseous components, including carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, and hydrocarbons. Particle size distributions were determined using both an impactor and an impactor/parallel flow diffusion battery. The actual particle concentration for the low-, medium-, and high-exposure levels were 0.353, 3.47, and 7.08 mg/m^3, respectively (duration-adjusted concentrations were 0.0735, 0.723, and 1.47 mg/m^3 for the low, medium, and high exposures, respectively). Mass median diameters (geometric standard deviations) determined using a impactor/parallel flow diffusion battery were 0.262 (4.2), 0.249 (4.5), and 0.234 (4.4) for the low-, medium-, and high-exposure groups, respectively. Lung wet weight to dry weight ratio was increased significantly in the two highest exposure groups. Qualitative descriptions of the histological results in the respiratory tract are found in Mauderly *et al.* (1987, 1988), Henderson *et al.* (1988), and McClellan *et al.* (1986). Aggregates of particle-laden macrophages were seen after 6 months in rats exposed to 7 mg/m^3 target concentrations, and after 1 year of exposure, histological changes were seen, including focal areas of epithelial metaplasia. Fibrosis and metaplasia increased with increasing duration of exposure and was observable in the 3.5 and 7 mg/m^3 group of rats at 24 months. Changes in the epithelium included extension of bronchiolar cell types into the alveoli. Focal thickening of the alveolar septa was also observed. Histological effects were seen in areas near aggregations of particle-laden macrophages. The severity of inflammatory responses and fibrosis was directly related to the exposure level. In the 0.35 mg/m^3 group of rats, there was no inflammation or fibrosis. Although the mouse lungs contained higher lung burdens of diesel particles per gram of lung weight at each equivalent exposure concentration, there was substantially less inflammatory reaction and fibrosis than was observed in rats. Fibrosis was observed only in the lungs of mice exposed at 7 mg/m^3 and consisted of fine fibrillar thickening of an occasional alveolar septa.

Groups of 16 rats and mice (8/sex) were subjected to bronchoalveolar lavage after 6, 12, 18, and 24 (rats only) months of exposure as described above (Henderson *et al.*, 1988). Lung wet weights were increased at 7 mg/m^3 in mice and rats at all time points and in mice at 3.5 mg/m^3 at all time points after 6 months. An increase in lavagable neutrophils indicating an inflammatory response in the lung was seen at 3.5 and 7 mg/m^3 in rats and mice at most time points. An increase in protein content of the bronchoalveolar lavage fluid was observed in rats exposed to

3.5 or 7 mg/m^3 at 12 and 18 months, but not at 24 months. Increased protein content was also seen in mice at the two higher concentrations at all time points. Increases in lavage fluid content of lactate dehydrogenase, glutathione reductase, beta-glucuronidase, glutathione, and hydroxyproline were observed in rats and mice exposed to 3.5 or 7 mg/m^3 at various time points. Analysis of lung tissue indicated changes in enzyme levels as well as an increase in total lung collagen content. Rats exposed for 24 months to diesel exhaust (3.5 mg/m^3 particulate matter) had a five-fold increase in the bronchoconstrictive prostaglandin PGF2-alpha and a two-fold increase in the inflammatory leukotriene LTB4. In similarly exposed mice, there was a two-fold increase in both parameters. These parameters were not measured in the other exposure groups. At the lowest exposure level, no biochemical or cytological changes occurred in the lavage fluid or in lung tissue in either Fischer 344 rats or CD-1 mice.

Mauderly *et al.* (1988; see also McClellan *et al.*, 1986) examined the impairment of respiratory function in rats exposed according to the protocol described above. After 12 months of exposure to the highest concentration of diesel exhaust, the exposed rats (n = 22) had lower total lung capacity (TLC), dynamic lung compliance (Cdyn), functional vital capacity (FVC), and carbon monoxide (CO) diffusing capacity than controls (n = 23). After 24 months of exposure to 7 mg/m^3 particulate matter, mean TLC, Cdyn, quasistatic chord compliance, and CO diffusing capacity were significantly lower than control values. Nitrogen washout and percentage of FVC expired in 0.1 second were significantly greater than control values. There was no evidence of airflow obstruction. Similar functional alterations were observed in the rats exposed to 3.5 mg/m^3 particulate matter, but such changes usually occurred later in the exposure period and were generally less pronounced. There were no significant decrements in pulmonary function for the 0.35 mg/m^3 group at any time during the study. This study demonstrates a LOAEL of 3.5 mg/m^3 (duration-adjusted concentration = 0.723 mg/m^3) and a NOAEL of 0.35 mg/m^3 (duration-adjusted concentration = 0.0735 mg/m^3) from a chronic study in rats and mice for respiratory effects including histological, functional, and biochemical parameters. The human equivalent exposure for the rat study was from the deposition and retention model developed by Yu and collaborators (Yu and Yoon, 1990). The LOAEL(HEC) and NOAEL(HEC) obtained in this way are 0.36 and 0.042 mg/m^3, respectively. The deposition and retention model is available only for rats at this time.

Wolff *et al.* (1987) investigated alterations in particle clearance from the lungs of rats chronically exposed to diesel exhaust at 0, 0.35, 3.5, or 7 mg/m^3 particulate matter for 7 hours/day, 5 days/week for up to 24 months. Progressive increases in lung burdens were observed over time in the 3.5- and 7-mg/m^3 exposure groups. Levels of diesel particles in terms of milligrams per lung were 0.60, 11.5, and 20.5 after 24 months of exposure at the 0.35-, 3.5-, or 7-mg/m^3 exposure levels, respectively. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of gallium oxide [0.1 μm mass median diameter (MMD)] as early as 6 months at the 7-mg/m^3 level and 18 months at the 3.5-mg/m^3 level; no significant changes were seen at the 0.35-mg/m^3 level. Rats inhaled fused aluminosilicate particles (2 μm MMAD) radiolabeled with cesium after 24 months of diesel exhaust exposure; long-term clearance half-times were 79, 81, 264, and 240 days for the 0-, 0.35-, 3.5-, and 7-mg/m^3 groups, respectively. Differences were significant between the control and the 3.5- and 7-mg/m^3 groups ($p<0.01$). The

LOAEL and NOAEL for effects on particle clearance identified in this study were identical to the LOAEL and NOAEL for respiratory effects identified in other results from the Inhalation Toxicology Research Institute (ITRI). No effect was seen on tracheal clearance of a labeled particle instilled onto the distal trachea or on the fast clearance component of an inhaled particle.

The dosimetric model uses the actual exposure duration and concentrations as inputs, so the duration-adjusted concentration is not used. The duration-adjusted concentration is calculated and presented only to allow comparison with studies for which the dosimetric model was not applied.

Both the ITRI and HERP studies identify a LOAEL and a NOAEL for pulmonary effects in rats. The exposure level to be used for derivation of the RfC is the highest NOAEL which is lower than all LOAELs for the same effect. Based on this criterion, the NOAEL from Ishinishi *et al.* (1988) [0.46 mg/m^3; NOAEL(HEC) = 0.155 mg/m^3] was selected.

Using the data on deposition and retention of diesel particles in laboratory animals as well as theoretical and empirical information on human deposition and retention of inhaled particles, a mathematical model has been developed that simulates these processes and can be used to extrapolate between rats and humans (Yu and Yoon, 1990; U.S. EPA, 1993). Using deposition and retention data for inhaled diesel particles in the rat, a retained dose per gram of lung or per unit epithelial surface can be calculated for each of the exposure scenarios for the different rat studies. The retention model takes into account the retardation of particle clearance due to the particle overload effect. Assuming that the long-term retained dose must be the same in rats and in humans to induce the same effect, a deposited dose for the human lung can be calculated from the retained dose applying human-specific retention half-times to arrive at the HEC. The retention model used by Yu and Yoon (1990) includes the three-compartment lung respiratory tract model with additional compartments for transport to blood, lymphatics, and the gastrointestinal tract. Diesel particles consist of a carbon core (80% of the total mass) and adsorbed organics. Half of the organic material is strongly bound to the core and half is weakly bound. The presence of two different organic phases is used based on empirical observations of biphasic clearance of diesel-particle-associated organics. Transport due to dissolution of the organic phase is assumed to be constant. All other transport processes are modeled using first-order rate constants, with the exception of the mechanical clearance of the carbon core from the alveolar compartment. Diesel particle transport from the alveolar compartment varies with particle lung burden after lung burden reaches a certain level. This has been referred to as the particle overload effect and has been observed with particles other than diesel (Morrow, 1992). At low particle burdens, the clearance rate was essentially normal, and at very high particle burdens, alveolar macrophage mediated clearance ceases completely. The functional dependence of mechanical alveolar clearance rates on particle lung burden used in the model was determined by fitting the experimental data in rats. The model mathematically describes deposition and transport of the three particle components (carbon core, weakly bound organics, and strongly bound organics) between compartments.

The noncancer toxicity of diesel emissions is considered to be due to the insoluble carbon core because the long-term effects seen with whole diesel are not found or are found to a much lesser

extent in laboratory animals exposed to similar dilutions of diesel exhaust filtered to remove most of the particles. In a manner consistent with the procedures described in U.S. EPA (1990), the human equivalent concentration is calculated for diesel particles based on the assumption that the equivalent dose metric across species is the retained mass per surface area in the alveolar (pulmonary) region. Because the dependence of mechanical alveolar clearance on particle lung burden in humans is not known, it was assumed in development of the model for humans that the particle overload phenomenon occurs in humans and in rats at equivalent lung burdens expressed as mass per unit surface area (Yu and Yoon, 1990). This assumption allows for the development of a diesel-particle-specific human retention model and therefore allows extrapolation from the rat studies to human exposures. The model has not been extended to other species at this time because data describing the dependence of the particle overload phenomenon on lung particle burden for species other than the rat are not available. Calculated human equivalent concentrations for rat studies are based on the model of Yu and Yoon (1990).

UNCERTAINTY AND MODIFYING FACTORS

UNCERTAINTY FACTORS:

The uncertainty factor of 30 reflects a factor of 10 to protect sensitive individuals and a factor of 3 to adjust for interspecies extrapolation because dosimetric adjustments based on a particle deposition and retention model were applied.

MODIFYING FACTORS: None

ADDITIONAL STUDIES / COMMENTS

Chronic inhalation studies using male Fischer 344 rats and male Hartley guinea pigs were conducted at the General Motors Research Laboratories (Barnhart *et al.*, 1981, 1982). Exposures to target concentrations of 0.25, 0.75, and 1.5 mg/m^3 were generated using a 5.7 L engine run at constant speed and load conditions (Schreck *et al.*, 1981). Constant operating conditions were used to achieve a more uniform exposure atmosphere. The number of animals exposed per group varied because several different studies were included in the exposure design. Exposures were for 20 hours/day, 5.5 days/week for a reported total of 110.5 hours/week for up to 2 years. Particle concentrations were determined with approximately 20 filter samples/week from each exposure level. Actual exposure concentrations were 0.258, 0.796, and 1.533 mg/m^3 (duration adjusted concentrations were 0.17, 0.52, and 1 mg/m^3, respectively). Particle size was determined weekly using a seven-stage cascade impactor. A diffusion battery was also developed to measure diffusional properties of the diesel particulate matter. Mass median aerodynamic diameter was reported to be 0.19 μm (Soderholm, 1980), and the geometric standard deviation was not reported in any of the published accounts of this study. Studies performed using the animals exposed under these conditions included studies of respiratory tract histology and ultrastructure, pulmonary function, particle transport, and lavaged cells. Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 months were reported by Barnhart *et al.* (1981).

In a continuation of these studies, guinea pigs were exposed to target concentrations of 0.25, 0.75, and 1.5 mg/m^3 of diesel exhaust for 2 years (Barnhart *et al.*, 1982). The focus of these studies is on electron micrographic morphometry, and very little descriptive light microscopic histology is described. Quantitative morphometric analysis showed that the alveolar-capillary membrane increased in thickness as a result of an increase in the absolute tissue volume of interstitium and Type II cells. Exposure of guinea pigs to 0.75 mg/m^3 for 6 months resulted in fibrosis in regions of macrophage clusters and in focal Type II cell proliferation observable by light microscopy. No additional information was provided regarding light microscopic evaluation of the fibrotic changes with increasing concentration or duration of exposure. There was increased cellular composition of the interstitium in animals exposed to 0.75 or 1.5 mg/m^3 consisting of a variety of inflammatory cell types. Hypertrophy and proliferation of Type II cells were observed as early as 2 weeks at 0.75 mg/m^3 or higher in guinea pigs. Mean thickness of the air-blood barrier remained elevated in the animals exposed to 0.75 and 1.5 mg/m^3, although the peak thickness occurred at 6 months to 1 year of exposure. At the concentration of 0.25 mg/m^3, after 9 months of exposure, there was a slight but not significant increase in Type I and II cells, endothelial cells, and interstitial cells over concurrent age-matched controls. These data show that no appreciable changes in morphometric parameters occurred after 2-year exposure to 0.25 mg/m^3, whereas exposure to 0.75 or 1.5 mg/m^3 resulted in increased thickness of alveolar septa and increased number of various types of alveolar cells.

Additional biochemical and cytological studies (Misiorowski *et al.*, 1980; Eskelson *et al.*, 1981; Strom, 1984) were conducted on rats exposed under the same conditions as reported on in the Barnhart *et al.* (1982) principal study. Increased numbers of polymorphonuclear leukocytes and monocytes lavaged from rats exposed to 0.75 or 1.5 mg/m^3 indicated an inflammatory response at these levels (Strom, 1984). In most cases, exposures to 0.25 mg/m^3 did not cause any significant changes. Misiorowski *et al.* (1980) reported on biochemical effects in rats exposed to 0.25 and 1.5 mg/m^3. DNA content in lung tissue and the rate of collagen synthesis were significantly increased after exposure to 1.5 mg/m^3 particulate matter for 6 months. Collagen deposition was not affected. Total lung collagen content increased in proportion to the increase in lung weight. After 9 months of exposure, there were significant increases in lung phospholipids in rats and guinea pigs exposed to 0.75 mg/m^3 and in lung cholesterol in rats and guinea pigs exposed to 1.5 mg/m^3 (Eskelson *et al.*, 1981). These studies demonstrate a LOAEL of 0.796 mg/m^3 and a NOAEL of 0.258 mg/m^3 for male guinea pigs in a chronic study for respiratory endpoints including light and electron microscopy, lavage cytology, and lung tissue biochemistry. Duration-adjusted LOAEL and NOAEL are 0.52 and 0.17 mg/m^3, respectively.

Kaplan, *et al.* (1982) reported on a subchronic study in which male Fischer 344 rats, A/J mice, and Syrian golden hamsters were exposed for 20 hours/day, 7 days/week for 3 months to diesel exhaust containing 1.5 mg/m^3 particulate matter. Diesel exposures were generated using a 5.7 L engine operated continuously for 20 hours/day, 7 days/week. Actual particle concentrations are not reported in the study and are assumed to be 1.5 mg/m^3 (duration-adjusted concentration is 1.25 mg/m^3). Particle size measurements are not reported for the subchronic study. The total number of animals used was not reported. Thirty control and exposed animals per species were used for necropsy and histological examination of 20 tissues, including three sections of the nasal cavity. Additional animals were used for ultrastructure and morphometry (number not specified)

and for a recovery study (30 animals/group/species). No effect on body weight was observed, and organ weights were not affected, except for a slight increase in the lung-to-body weight ratio of rats. Microscopic examination revealed no anatomic changes in the upper respiratory tract. Most of the particles were in macrophages, but some were free as small aggregates on alveolar and bronchiolar surfaces. The particle-laden macrophages were often in masses in respiratory ducts. A minimal increase in the thickness of the alveolar walls was associated with masses. After 6 months of recovery, the lungs of all three species contained considerably less pigment, as assessed by gross pathological and histopathological examinations.

A 15-month inhalation study was performed by Southwest Research Institute for General Motors (Kaplan *et al.*, 1983). Male Fischer 344 rats, Syrian golden hamsters, and A/J mice were exposed to diluted diesel exhaust at target concentrations of 0.25, 0.75, and 1.5 mg/m^3 for 20 hours/day, 7 days/week. Diesel emissions were generated using a 5.7 L engine operated continuously at a speed and load that simulates a speed of 40 mph. The exhaust was transported directly to the chamber in heated lines, and dilution occurred just prior to entry into the chamber. Particle concentration was measured daily using a filter, and particle size distribution was measured weekly using a seven-stage cascade impactor. Actual particle concentrations were 0.242, 0.735, and 1.500 mg/m^3 (duration-adjusted concentrations were 0.202, 0.613, and 1.25 mg/m^3), respectively. The MMAD and sigma g were not reported, but the MMAD appears to fall between 0.2 and 0.25 μm based on the graphical presentation of the size distribution data, and 88-93% of the particles were reported to be less than 1 μm in aerodynamic diameter. Histopathological examinations were performed on 50 rats and hamsters and 30 mice after 9 months of exposure, and on 30 rats and hamsters after 15 months. The respiratory tract, including larynx, main bronchi, lungs, and three sections of the nasal cavity, was examined in all animals. Histological examination of 18 other tissues was performed on 20 animals from the control and high-dose groups only. Groups of 10 rats and hamsters/exposure level were also used for electron microscopic examination at 9 and 15 months of exposure, and 10 mice per exposure level were used for this purpose at 9 months of exposure. Most of the mice were used to evaluate the effect of diesel exposure on development of pulmonary adenomas after 7 months of exposure. No exposure-related effect on body weight or survival was observed in any species. No effects were found in the upper respiratory tract of any species. After 9 months of exposure, most rats showed perivascular accumulations of lymphocytes, and some showed foci of pneumonitis. These lesions were not exposure related and may indicate the presence of lung disease in the animals. Concentration-related increases in pigment deposition observed in rats, mice, and hamsters were considered minimal in the 0.250 mg/m^3 group and increased in severity with increasing concentration. The focal accumulation of particle-laden macrophages was associated with minimal to mild fibrosis of the alveolar wall. This lesion was reported to be more severe in the high concentration group, but was not reported as a separate pathological entity; rather, the particle accumulation and associated macrophage and epithelial effects were combined under the term pneumoconiosis, and only the incidence of this entity was reported. No exposure-related lesions were found in tissues other than the respiratory tract. After 15 months of exposure, histopathological results in rats were confounded by the presence of focal chronic pneumonia and lymphocyte accumulation in control and exposed rats. As in the 9-month animals, the incidence of pneumoconiosis is reported to increase in severity with increasing

exposure concentration. It is not clear whether this term is applied only to particle accumulation or whether associated epithelial damage is included. Epithelial effects are not described after 15 months. This study does not clearly identify a LOAEL for epithelial histopathology because it is not clear whether pathological epithelial effects occurred under the conditions of the study. Based on particle-laden macrophage accumulation, this study identifies a LOAEL at 0.735 mg/m^3 and a NOAEL at 0.242 mg/m^3 in rats, mice, and hamsters.

In a study performed by NIOSH (Lewis *et al.*, 1986, 1989; Green *et al.*, 1983), male and female Fischer 344 rats and male cynomolgus monkeys were exposed to diesel emissions. A total of 288 rats/sex/exposure and 15 monkeys/exposure were exposed for 24 months, and an additional 144 male rats/exposure were used for interim sacrifices at 3, 6, 12, and 24 months. The target exposure level was 2 mg/m^3 particulate matter for 7 hours/day, 7 days/week and was generated from a 7 L engine operated on a load cycle intended to simulate use in mining. Particle concentrations were measured daily using filter samples. Actual exposure concentrations were 1.95 mg/m^3 (duration-adjusted concentration of 0.57 mg/m^3). Particle size distribution was determined using an electric aerosol analyzer and by sampling on a 0.1 μm filter, followed by scanning electron microscopic (SEM) analysis. The MMDs were 0.23 μm by the instrumental technique and 0.36 μm by the SEM approach, and the sigma g was 2.5 and 2 for the instrumental and SEM approaches, respectively. In rats, accumulations of black-pigmented alveolar macrophages were seen in the alveolar ducts adjacent to terminal bronchioles as early as 3 months of exposure, and particles were seen within the interstitium of the alveolar ducts. These lesions increased in size up to 12 months of exposure. Collagen or reticulum fibers were seen only rarely in association with deposited particles; the vast majority of lesions showed no evidence of fibrosis. Multifocal histiocytosis (24% of exposed rats) was observed only after 24 months of exposure. These lesions were most commonly observed subpleurally and were composed of collections of degenerating macrophages and amorphous granular material within alveoli, together with fibrosis and chronic inflammatory cells in the interstitium. Epithelial lining cells adjacent to collections of pigmented macrophages showed a marked Type II cell hyperplasia; degenerative changes were not observed in Type I cells. Histological examination of lung tissue from monkeys exposed for 24 months in the same regimen as used for rats revealed aggregates of black particles, principally in the distal airways of the lung. Particles were present within the cytoplasm of macrophages in the alveolar spaces as well as the interstitium. Fibrosis, focal emphysema, or inflammation was not observed. Lewis *et al.* (1989) evaluated 10 control and 10 diesel-exposed rats (2 mg/m^3 particulate matter, 7 hours/day, 5 days/week for 52 or 104 weeks) for responses in functional residual capacity and airway resistance and conductance. At the 104-week evaluation, the rats were also examined for maximum flow volume impairments. No evidence of an impairment of pulmonary function as a result of the exposure to diesel exhaust was found in rats. Diesel-exhaust-exposed monkeys were evaluated prior to exposure and at 6-month intervals up to 24 months for pulmonary compliance and resistance, all static and dynamic lung volumes, diffusing capacity, distribution of ventilation, and maximal ventilatory performance (flow and volume). The monkeys exposed to diesel exhaust demonstrated small airway obstructive disease. The obstructive impairment was most detectable using the forced expiratory flow at 40% (FEF40) of the TLC instead of the FEF as a percentage of the vital capacity. This significant finding is indicative of a shift in the flow

volume curve as a result of mild hyperinflation. This study demonstrates a LOAEL for rats and monkeys at a duration-adjusted diesel particle concentration of 0.57 mg/m^3. The LOAEL(HEC) for rats estimated from the retention model of Yu and Yoon (1990) for these exposure conditions is 0.346 mg/m^3.

Iwai *et al.* (1986) performed serial histopathology on the lungs of female Fischer 344 rats at 1, 3, 6, 12, and 24 months of exposure to diesel exhaust (24 animals/group). Exposures were for 8 hours/day, 7 days/week for 24 months; the exposure atmosphere contained 4.9 mg/m^3 particulate matter (duration-adjusted concentration of 1.63 mg/m^3). The sampling method, duration, number of samples, and particle size distribution were not reported. Exposures were generated using a 2.4 L engine operated at constant load. Body weights were measured biweekly, and at the end of the exposure, animals were examined using light and electron microscopy. At 1 and 3 months of exposure, there were minimal histological changes in the lungs of the exposed rats. After 6 months of exposure, there were particle-laden macrophages distributed irregularly throughout the lung and a proliferation of Type II cells with adenomatous metaplasia in areas where the macrophages had accumulated. After 1 year of exposure, foci of hyperplasia of the ciliated or nonciliated bronchiolar epithelium on the adjacent alveolar walls were more common, the quantity of deposited particulate matter increased, and the number of degenerative alveolar macrophages and proliferative lesions of Type II or bronchiolar epithelial cells increased. After 2 years of exposure, there was a fibrous thickening of the alveolar walls, mast cell infiltration with epithelial hyperplasia in areas where the macrophages had accumulated, and neoplasms.

Heinrich *et al.* (1986; see also Stoeber, 1986) exposed male and female Syrian golden hamsters, female NMRI mice, and female Wistar rats to diesel engine emissions with a target 4-mg/m^3 particulate concentration (96 animals/group). Diesel emissions were generated using a 1.6 L engine operated on a standard driving cycle. Actual particle concentrations were determined by filter samples (number of samples not specified) to be 4.24 mg/m^3. Particle size distribution determined using a 10-stage impactor (number of samples not specified) was 0.35 um. Exposures were for 19 hours/day, 5 days/week (duration-adjusted concentration of 2.40 mg/m^3); the maximum exposure period was 120 weeks for hamsters and mice and 140 weeks for rats. Animals were examined after 1 or 2 years of exposure for lung weights; alveolar lung clearance (3, 8, 12, and 19 months of exposure); lung function; biochemistry and cytology of lung lavage fluid; and histopathology on 19 tissues, including the nasal cavity, larynx, and trachea. Lung weights were increased by a factor of 2 or 3 in rats and mice after 2 years of exposure, and in hamsters, the lung weights were increased by 50-70%. Histological examination revealed different levels of response among the three species. In hamsters, the exhaust produced thickened alveolar septa and bronchioalveolar hyperplasia. In mice, bronchioalveolar hyperplasia occurred in 64% of the mice exposed to the exhaust and in 5% of the controls. Multifocal alveolar lipoproteinosis occurred in 71%, and multifocal interstitial fibrosis occurred in 43% of the mice exposed to exhaust, but in only 4% of the controls. No effects were seen in the nose, larynx, or trachea. After 1 year of exposure to the diesel exhaust, the hamsters exhibited a significant increase in airway resistance and a nonsignificant reduction in lung compliance. These changes did not change during the second year of exposure. In exposed rats, there were severe inflammatory changes in the lungs, as well as thickened septa, foci of macrophages, and hyperplastic and metaplastic lesions. When compared with a control group,

no significant changes in respiratory rate, minute volume, compliance, or resistance occurred in the exposed rats (n = 14). Significantly increased airway resistance and a significant decrease in Cdyn were observed in exposed rats after 2 years of exposure. This study demonstrates a LOAEL(ADJ) in rats, mice, and hamsters for respiratory system effects of 2.4 mg/m^3.

The effects of diesel exhaust on the lungs of 18-week-old male Wistar rats exposed to 8.3 +/- 2 mg/m^3 particulate matter were investigated by Karagianes *et al.* (1981). Exposures were for 6 hours/day, 5 days/week, for 4, 8, 16, or 20 months (duration-adjusted concentration of 1.48 mg/m^3) and were generated using an HD diesel engine operated on a mining simulation pattern. The exposure concentration was obtained by diluting the diesel engine emissions as needed to achieve a CO level of 50 ppm. The method and number of samples for estimating particle concentration were not specified. Particle size distribution was measured using a cascade impactor. Diesel particulate matter had a MMAD of 0.71 μm and a geometric standard deviation of 2.3 (number of samples were not reported). Airflow through the diesel exposure chamber was low (50 L/minute), and chamber ammonia concentrations reached 26-40 ppm. Six animals/group were examined after 4, 8, 16, and 20 months of exposure. Histological examinations of a limited number of tissues, including nose, trachea, larynx, and lungs, were performed. Histological examinations of lung tissue noted focal aggregation of particle-laden alveolar macrophages, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema. Lesion severity was related to length of exposure. No exposure-related effects were seen in the nose, larynx, or trachea. This study demonstrates a LOAEL(ADJ) of 1.48 mg/m^3 for respiratory effects after chronic exposure of rats to diesel emissions.

The lung function of adult cats chronically exposed to diesel exhaust generated from a 3.24 L engine operated on a simulated driving cycle was measured (Pepelko *et al.*, 1980; Pepelko, 1982; Moorman *et al.*, 1985). The cats were exposed for 8 hours/day, 7 days/week for 120 weeks. Actual exposures determined from daily filter samples were 6.34 mg/m^3 for the first 61 weeks and 11.7 mg/m^3 for weeks 62-124 (duration-adjusted and time-weighted concentration = 3 mg/m^3). Particle size was determined using SEM measurement of particles collected on a filter. Mass median diameter was reported to be 0.3 um. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes (TLC, FVC, functional residual capacity, and inspiratory capacity) and the significantly lower single-breath diffusing capacity, coupled with normal values for dynamic ventilatory function, indicate the presence of a lesion that restricts inspiration but does not cause airway obstruction or loss of elasticity. This pulmonary physiological syndrome is consistent with an interstitial fibrotic response that was later verified by histopathology (Plopper *et al.*, 1983).

Brightwell *et al.* (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Three exhaust dilutions were generated from a 1.5 L engine operated according to a simulated driving cycle, producing concentrations of 0.7, 2.2, and 6.6 mg/m^3 particulate matter. The measurement method and number of samples used to determine the exposure concentration were not specified. No estimates of particle size distribution were reported. The test animals [144 rats and 312 hamsters (equal numbers of males and females)/exposure group] were exposed

for five 16-hour periods/week for 2 years (duration-adjusted concentrations = 0.33, 1.05, and 3.14 mg/m^3). Interim sacrifices of 16 animals/group were performed at 6, 12, 18, and 24 months in rats and at 6 and 16 months in hamsters. Surviving rats were examined at 30 months, and hamsters were examined at 24 months. Histopathology was performed on the nose, larynx, trachea, and lungs from control and high-concentration groups. Blood chemistry; hematology; urine chemistry; and respiratory physiology measurements including tidal volume, compliance, airway resistance, respiratory rate, inspiratory volume, FVC, expiratory reserve volume, peak flows, blood pressure, and heart rate were evaluated. Cardiovascular function was evaluated only in male rats at the 24-month sacrifice. Blood and urine analyses were performed at the interim sacrifices. Results were reported very briefly for the control and high-concentration groups only in this study, and actual values were not provided. Body weights were significantly lower in the rats exposed to 2.2 or 6.6 mg/m^3. There were no differences in the urinalysis between exposed and control animals. There were several blood chemistry and hematology parameters in rats exposed for 18 or 24 months and hamsters exposed for 16 months that were significantly different in exposed animals compared with controls. The respiratory physiology measurements were not affected in high-concentration hamsters, but were significantly changed in high-concentration rats. The changes in rats were not specified, but were summarized as being consistent with obstructive and restrictive disease in the high-concentration group. No detailed information on histopathological results or pulmonary function results were provided, and no further published accounts of this study could be located, except a discussion of the tumorigenic response in rats and hamsters (Brightwell *et al.*, 1989). Because only results from the high-concentration groups were reported, this study is not useful for RfC development. The high concentration was shown to be an adverse-effect level in rats and hamsters with a duration adjusted value of 3.1 mg/m^3. The results of the study with filtered diesel exhaust are discussed later (Brightwell *et al.*, 1986).

Takemoto *et al.* (1986) reported a chronic study in Fischer 344 rats, C57BL/6N mice, and ICR mice (sex not specified). Exposures were generated using a 0.27 L engine, and particle concentration was reported as 2-4 mg/m^3. The measurement method and number of samples were not discussed. The "mean diameter" was reported to be 0.32 um, but the measurement method was not specified. Exposures were conducted for 4 hours/day, 4 days/week for up to 24 months (duration-adjusted exposure concentration = 0.19-0.38 mg/m^3). Groups of animals were examined at 6, 12, 18, and 24 months in rats and mice and also at 3 months in mice. Lung tissue was examined using light and electron microscopy, and nasal sections were examined using light microscopy. Alveolar hyperplasia was increased in rats and mice at 12 months and later. Slight chronic inflammatory lesions were noted in the nose and lungs of the control group and were ascribed to the fact that animal housing was not in a barrier facility. Exposed rats were described as having more severe inflammation with inflammatory exudate into the nose, as well as bronchitis and pneumonia. Although these exposures are at levels lower than other studies, the effects are not well described and are not consistent with effects reported in other, better controlled studies, and these results are not suitable for derivation of an RfC.

Werchowski *et al.* (1980a) reported a developmental study in rabbits exposed on days 6-18 of gestation to a 1-in-10 dilution of diesel exhaust. The exposure protocol for this and other U.S. EPA studies was reviewed by Pepelko and Peirano (1983), and the target exposure level of 6

mg/m^3 was used. Exposure measurement and characterization of the particle size distribution at the time of the developmental study were not provided. At day 29 of gestation, laparotomy was performed; reproductive parameters were determined; and fetuses were examined for external, internal, and skeletal abnormalities. Exposure to diesel emissions had no effect on maternal toxicity or on the developing fetuses (NOAEL = 6 mg/m^3). In a companion study by Werchowski *et al.* (1980b), 20 SD rats were exposed for 8 hours/day during days 5-16 to a target concentration of 6 mg/m^3 of diesel particles (Pepelko and Peirano, 1983). Exposure measurement and characterization of the particle size distribution at the time of the developmental study were not provided. Fetuses were examined for external, internal, and skeletal malformations and number of live and dead fetuses, resorptions, implants, corpora lutea, fetal weight, litter weight, sex ratio, and maternal toxicity were recorded. No evidence of developmental effects was observed in this study (NOAEL = 6 mg/m^3).

In a U.S. EPA-sponsored reproductive study summarized by Pepelko and Peirano (1983), CD-1 mice were exposed to a target concentration of 12 mg/m^3 for 8 hours/day, 7 days/week (duration-adjusted concentration = 4 mg/m^3). The exposure generation equipment was as described for the Pepelko (1982) study. Measurements of exposure concentration and particle size performed at the time of the reproductive study were not specified by Pepelko and Peirano (1983). The F0 and F1 animals were exposed for 100 days prior to breeding, and 100 mating pairs were randomly assigned to four exposure groups of 25 pairs each. Viability counts and pup weights were recorded at 4, 7, and 14 days after birth and at weaning. Male and female fertility indices, pup sex ratio, pup survival indices, parental body weights, pup body weights, organ weights, and number of live births were recorded. A decrease in body weight in unmated F1 females was noted, but data were not provided. No treatment-related effects on body weight were found in F0 mice, in F1 animals through weaning, or in mating animals through gestation. No treatment-related effects on gestation length, percent fertile, litter size, or pup survival were observed. The only organ weight difference was an increase in lung weight in exposed F0 and F1 mice (lung weight and lung weight/body weight) and in F2 males (lung weight/body weight). Based on this study, a NOAEL(ADJ) for reproductive and limited developmental effects in rats is identified at 4 mg/m^3. Several laboratory animal studies have been performed to compare the effects of exposure to whole exhaust with the effects of filtered exhaust containing no particles. These studies demonstrate that when the exhaust is sufficiently diluted to limit the concentrations of gaseous irritants, the diesel particles are the prime etiologic agents of noncancer health effects, although additivity or synergism with the gases cannot be ruled out. The gas phase of diesel exhaust contains nitrogen oxides (NOx), sulfur oxides, aldehydes, CO, hydrocarbons, and polycylic aromatic hydrocarbons (PAHs). In the NIOSH study, at a concentration resulting in 2 mg/m^3 particles, the gas phase contained 11.5 ppm CO, 1.5 ppm nitrogen dioxide (NO2), 0.81 ppm sulfur dioxide (SO2), 0.04 ppm formaldehyde, 0.06 ppm acrolein, 7.5 ppm total hydrocarbons, and 300 ug/m^3 of five different PAHs. The whole-diesel exposures produced changes in the lung that are much more prominent than those evoked by the gas phase alone. Such marked differences between whole and filtered diesel exhaust are evident from general toxicological indices, such as decreases in body weight and increases in lung weights, pulmonary function measurements, and pulmonary histopathology. Based on these results, the derivation of the RfC is based on the dose of the particulate phase to the lung surface.

Heinrich *et al.* (1986) compared the toxic effects of whole and filtered diesel exhaust on hamsters, rats, and mice using the exposure protocol described previously. Body weights of rats and mice were reduced by the whole exhaust, but not by the filtered exhaust. After 1 year of exposure to the whole exhaust, hamsters exhibited increased lung weights, a significant increase in airway resistance, and a nonsignificant reduction in lung compliance. For the same time period, rats exhibited increased lung weights, a significant decrease in Cdyn, and a significant increase in airway resistance. Test animals exposed to filtered exhaust did not exhibit such effects. Histopathological examination indicated that different levels of response occurred in the three species. In hamsters, filtered exhaust caused no significant histopathological effects in the lung; whole exhaust caused thickened alveolar septa, bronchioalveolar hyperplasia, and emphysematous lesions. In mice, whole exhaust, but not filtered exhaust, caused multifocal bronchioalveolar hyperplasia, multifocal alveolar lipoproteinosis, and multifocal interstitial fibrosis. In rats, there were no significant morphological changes in the lungs following exposure to filtered exhaust. In rats exposed to whole exhaust, there were severe inflammatory changes in the lungs, thickened alveolar septa, foci of macrophages, crystals of cholesterol, and hyperplastic and metaplastic lesions. Biochemical studies of lung lavage fluids of hamsters and mice indicated that exposure to filtered exhaust caused fewer changes than did exposure to whole exhaust. The latter produced significant increases in lactate dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), total protein, protease (pH 5.1), and collagen. The filtered exhaust had a slight, but nonsignificant, effect on G6P-DH, total protein, and collagen. Similarly, cytological studies showed that, although the filtered exhaust had no effect on differential cell counts, the whole exhaust resulted in an increase in leukocytes (161 +/- 43.3/μL vs. 55.7 +/- 12.8/μL in the controls), a decrease in macrophages (30 +/- 12.5/μL vs. 51.3 +/- 12.5/μL in the controls), and an increase in granulocytes (125 +/-39.7/μL vs. 1.23 +/- 1.14/μL in the controls). All values presented for this study are the mean with its standard deviation. The differences were significant for each cell type. There was also a small increase in lymphocytes (5.81 +/- 4.72/μL vs. 3.01 +/- 1.23/μL in the controls).

Iwai *et al.* (1986) exposed rats (24/group) to whole or filtered diesel exhaust. The whole exhaust was diluted to achieve a concentration of 4.9 g/m^3 particulate matter. Body weights in the whole-exhaust group began to decrease after 6 months and, in both exposed groups, began to decrease after 18 months, when compared with controls. Lung-to-body weight ratios of the rats exposed to the whole exhaust showed a significant increase ($p < 0.01$) after 12 months in comparison with control values. After 6 months of exposure to whole exhaust, particles accumulated in alveolar macrophages, and Type II cell hyperplasia was observed. After 2 years of exposure to whole exhaust, the alveolar walls had become fibrotic with mast cell infiltration and epithelial hyperplasia. In contrast, rats exposed to filtered exhaust showed only minimal histologic changes in the lungs, with slight hyperplasia and stratification of bronchiolar epithelium and infiltration of atypical lymphocytic cells in the spleen, after 2 years.

Brightwell *et al.* (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Although detailed results are not provided, the inference from the discussion section of the paper was that there was a minimum of toxicity in the animals exposed to filtered diesel exhaust.

Diesel particulate matter is composed of an insoluble carbon core with a surface coating of relatively soluble organic constituents. Studies of diesel particle composition have shown that the insoluble carbon core makes up about 80% of the particle mass and that the organic phase can be resolved into a more slowly dissolving component and a more quickly dissolving component. Because macrophage accumulation, epithelial histopathology, and reduced clearance have been observed in rodents exposed to high concentrations of chemically inert particles (Morrow, 1992), it appears possible that the toxicity of diesel particles results from the carbon core rather than the associated organics. However, the organic component of diesel particles consists of a large number of PAHs and heterocyclic compounds and their derivatives. A large number of specific compounds have been identified. These components of diesel particles may also be responsible for the pulmonary toxicity of diesel particles. It is not possible to separate the carbon core from the adsorbed organics in order to compare the toxicity. As an approach to this question, a study has been performed at the Lovelace Inhalation Toxicology Research Institute in which rats were exposed to either diesel exhaust or to carbon black, an inert analog of the carbon core of diesel particles. Rats were exposed for 16 hours/day, 5 days/week, for up to 24 months to either 2.5 or 6.5 mg/m^3 of the particle (duration-adjusted concentrations = 1.2 and 3.1 mg/m^3). This study has not been completed, but a preliminary report is provided in the ITRI Annual Report for 1991 (Nikula *et al.*, 1991). Although the study is primarily concerned with the role of particle-associated organics in the carcinogenicity of diesel exhaust, nonneoplastic effects are also mentioned. According to the preliminary report, both diesel exhaust and carbon black exposure resulted in macrophage hyperplasia, epithelial hyperplasia, bronchioalveolar metaplasia, and focal fibrosis. Although the analyses have not yet been completed, the preliminary report states that the number and intensity of the lesions seem to correspond to the exposure time and concentration and that the morphological characteristics of the lesions were similar in the animals exposed to diesel and to carbon black. The preliminary results suggest that the chronic noncancer effects of diesel exhaust exposure are caused by the persistence of the insoluble carbon core of the particles, rather than by the extractable organic layer. On this basis, the derivation of the RfC is based on the calculation of the human equivalent dose with the retained mass of the carbon core per unit of pulmonary surface area as the dose metric that is considered equivalent across species.

There is a substantial body of evidence for an impairment of particulate clearance from the bronchioalveolar region of rats following exposure to diesel exhaust. The phenomenon of particle overloading in the lungs has also been observed in animals with exposure to other particulate substances (Morrow, 1992). Griffis *et al.* (1983) exposed rats 7 hours/day, 5 days/week for 18 weeks to diesel exhaust at 0.15, 0.94, or 4.1 mg/m^3 particulate matter. Lung burdens of the 0.15, 0.94, and 4.1 mg/m^3 levels were 35, 220, and 1890 ug/g lung, respectively, 1 day after the 18-week exposure. The clearance half-time of the diesel particles was significantly greater, almost double, for the 4.1-mg/m^3 exposure group than for those of the lower exposure groups, 165 +/-8 days versus 99 +/- 8 days (0.94 mg/m^3) and 87 +/- 28 days (0.15 mg/m^3), respectively. This study identifies a LOAEL of 4.1 mg/m^3 (duration-adjusted LOAEL = 0.85 mg/m^3) and a NOAEL of 0.94 mg/m^3 (duration-adjusted NOAEL = 0.20 mg/m^3) for effects on pulmonary clearance.

Chan *et al.* (1984) showed a dose-related slowing of 14C-diesel particle clearance in rats preexposed to diesel exhaust at 0.25 or 6 mg/m^3 particulate matter for 20 hours/day, 7 days/week for 7-112 days. Clearance was inhibited in the 6-mg/m^3 group at 62 and 112 days. No effect on clearance was observed in the 0.25-mg/m^3 rats at 52 and 112 days of exposure. In this study, a LOAEL of 6 mg/m^3 (duration-adjusted LOAEL = 5.1 mg/m^3) and a NOAEL of 0.25 mg/m^3 (duration-adjusted NOAEL = 0.21 mg/m^3) for effects on particle clearance were identified.

Heinrich *et al.* (1982) evaluated lung clearance in rats exposed for approximately 18 months at 3.9 mg/m^3 particulate matter for 7-8 hours/day, 5 days/week. Following exposure to radiolabeled iron oxide aerosol, the rats were returned to the diesel exhaust exposure, and the radioactivity was measured over the thoracic area at subsequent times. The biological half-life of the iron oxide deposited in the rats' lungs was nearly twice that of controls. The LOAEL for particle clearance in this study was 3.9 mg/m^3 (duration-adjusted concentration = 0.87 mg/m^3).

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers. None of these studies are used as the basis for derivation of the RfC because of inadequate exposure characterization. Battigelli *et al.* (1964) measured several indices of pulmonary function, including vital capacity, forced expiratory volume in 1 second (FEV_1), peak flow, nitrogen washout, and diffusion capacity in 210 locomotive repairmen exposed to diesel exhaust in three engine houses. The average exposure of these locomotive repairmen to diesel exhaust was 9.6 years. When compared with a control group matched for age, body size, "past extrapulmonary medical history" (no explanation given), and job status (154 railroad yard workers), no significant clinical differences were found in pulmonary function or in the prevalence of dyspnea, cough, or sputum between the diesel-exhaust-exposed and nonexposed groups. Exposure to the diesel exhaust showed marked seasonal variations because the doors of the engine house were open in the summer and closed in the winter. For the exposed group, the maximum daily workplace concentrations of air pollutants measured were 1.8 ppm NO_2, 1.7 ppm total aldehydes, 0.15 ppm acrolein, 4 ppm SO_2, and 5 ppm total hydrocarbons. The concentration of airborne particles was not reported.

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

Purdham *et al.* (1987) performed a cross-sectional evaluation of respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations that were exposed to both diesel and gasoline exhausts and in a control group of 11 on-site office workers. Twenty-four

percent of the exposed group and 36% of the controls were smokers. If a particular symptom was considered to be influenced by smoking, smoking status was used as a covariate in the logistic regression analysis; pack-years smoked was a covariate for lung function indices. The frequency of respiratory symptoms was not significantly different between the two groups; however, baseline pulmonary function measurements were significantly different. The latter comparisons were measured by multiple regression analysis using the actual (not percentage predicted) results and correcting for age, height, and pack-years smoked. The stevedores had significantly lower FEV_1, FEV_1/FVC, FEF_{50}, and FEF_{75} ($p<0.021$, $p<0.023$, $p<0.001$, and $p<0.008$, respectively), but not FVC. The results from the stevedores were also compared with those obtained from a study of the respiratory health status of Sydney, Nova Scotia, residents. These comparisons showed that the dock workers had higher FVC, similar FEV_1, but lower FEV_1/FVC and flow rates than the residents of Sydney. Based on these consistent findings, the authors concluded that the lower baseline function measurements in the stevedores provided evidence of an obstructive ventilatory defect, but caution in interpretation was warranted because of the small sample size. here were no significant changes in lung function over the work shift, nor was there a difference in lung function measurements between the two groups. The stevedores were exposed to significantly ($p<0.04$) higher concentrations of particulate matter (0.06-1.72 mg/m^3, mean of 0.50 mg/m^3) than the controls (0.13-0.58 mg/m^3, mean not reported). Based on 34 personal workshift samples for the exposed group, exposures of stevedores to SO_2, NO_2, aldehydes, and PAHs were very low; occasional CO concentrations in the 20-100-ppm range could be detected for periods up to 1 hour in areas where blockers were chaining gasoline-powered vehicles.

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

miner in this group had at least 1 year of underground mining work history in 1977. The control group was 838 miners with no exposure to diesel exhaust. The miners were evaluated for the prevalence of respiratory symptoms; chronic cough; phlegm; dyspnea; and changes in FVC, FEV_1, and FEF_{50}. No air monitoring data were reported; exposure conditions to diesel exhaust gases and mine dust particles were described as very low. These authors found no decrements in pulmonary function or increased prevalence of respiratory symptoms attributable to exposure to diesel exhaust. In fact, the 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than those exposed to diesel exhaust.

Attfield (1978) studied 2659 miners from 21 mines (8 metal, 6 potash, 5 salt, and 2 trona). Diesels were employed in only 18 of the mines, but those 3 mines not using diesels were unidentified. The years of diesel usage, ranging from 8 in trona mines to 16 in potash mines, were used as a surrogate for exposure to diesel exhaust. Based on a questionnaire, an increased prevalence of persistent cough was associated with exposure to aldehydes; this finding, however, was not supported by the pulmonary function data. No adverse respiratory symptoms or pulmonary function impairments were related to carbon dioxide, CO, NO_2, inhalable dust, or inhalable quartz. The author failed to comment on whether the prevalence of cough was related to the high incidence, 70%, of smokers in the cohort.

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

Gamble *et al.* (1983) investigated respiratory morbidity in 259 miners from five salt mines in terms of increased respiratory symptoms, radiographic findings, and reduced pulmonary function associated with exposure to NO_2, inhalable particles (<10 μm MMAD), or years worked underground. Two of the mines used diesel extensively; no diesels were used in one salt mine. Diesels were introduced into each mine in 1956, 1957, 1963, or 1963-1967, respectively. Several working populations were compared with the salt miner cohort. After adjustment for age and smoking, the salt miners showed no increased prevalence of cough, phlegm, dyspnea, or airway obstruction (FEV_1/FVC) compared with aboveground coal miners, potash miners, or blue-collar workers. The underground coal miners consistently had an elevated level of symptoms. Forced expiratory volume at 1 second, FVC, FEF_{50}, and FEF_{75} were uniformly lower for salt miners in relation to all the comparison populations. There was, however, no association between changes in pulmonary function and years worked, estimated cumulative inhalable particles, or estimated NO_2 exposure. The highest average exposure to particulate matter was 1.4 mg/m^3 (particle size not reported, measurement includes sodium chloride). Mean NO_2 exposure

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

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EPA CONTACTS	Daniel J. Guth / NCEA -- (919)541-4930 Annie M. Jarabek / NCEA -- (919)541-4847

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APPENDIX B

HEALTH EFFECTS OF AMBIENT PARTICULATE MATTER

Epidemiologic studies provide evidence for an association between ambient particulate matter and several adverse health outcomes including: mortality, respiratory and cardiac hospital admissions, emergency room visits, restricted activity days and respiratory symptoms for adults, lower respiratory illness for children, asthma attacks, and chronic disease. Among these studies, statistically significant relationships have been found using several alternative measures of particulate matter including TSP, PM_{10} (particles less than 10 microns in diameter) fine particles (particles less than 2.5 microns in diameter), British smoke, coefficient of haze (COH) and sulfates.

The studies have been conducted in several different cities and seasons, thereby incorporating a wide range of climates, chemical compositions of particulate matter, and populations. The reported epidemiologic investigations involve two principal study designs: time-series and cross-sectional. Time-series analysis examines changes in a health outcome within a specific area as air pollution levels fluctuate. A cross-sectional analysis compares differences in health outcomes across several cities at a selected point or period of time. The time-series studies have the distinct advantage of reducing or eliminating the problems associated with confounding or omitted variables, a common concern in the cross-sectional studies. Since the population characteristics are basically constant over the study period, the only factors that may vary with daily mortality and morbidity are environmental and meteorological conditions. In general, researchers are able to more easily elicit the effects of air pollution and weather on mortality using time-series analysis. However, by design, these studies only incorporate the effects of relatively short-term exposure.

MORTALITY

An association between particulate matter and mortality has been indicated from both time-series and cross-sectional studies. The time-series studies link daily counts of mortality in a metropolitan area with the ambient particle concentrations. These studies typically control for season, temperature, humidity, day of week, and other pollutants. Research has demonstrated associations on a daily basis between concentrations of particulate and mortality in several metropolitan areas including: London (Schwartz *et al*., 1990) Steubenville, Ohio (Schwartz *et al.*, 1992), Philadelphia (Schwartz *et al*., 1991), Santa Clara County (Fairley, 1990), Los Angeles (Shumway *et al*., 1988), Detroit (Schwartz 1991), Birmingham, AL (Schwartz 1993), Utah Valley (Pope *et al*., 1992) and Los Angeles (Kinney *et al*., 1991; Ostro, 1995). Several meta-analyses of these time-series studies suggests that, after converting the alternative measures of particulate matter used in the original studies to PM_{10}, the estimated effects on mortality are fairly consistent (Ostro, 1992; Schwartz, 1994; Pope *et al*., 1994). The U.S. Environmental Protection Agency confirmed these findings in their own assessment of the scientific evidence (U.S. Environmental Protection Agency, 1996).

Two types of studies exist that measure the impact of long-term exposure to particulate matter. The first type involves the use of a prospective cohort design in which a sample is selected and followed over time in each location published results (Dockery *et al*, 1993)for a 15-year prospective study based on samples of individuals in 6 cities, and published results (Pope *et al*., 1995) of a 7-year prospective study based on samples of individuals in 151 cities in the United States. These studies use individual-level data so that other health risk factors can be better characterized. Specifically, the authors of the prospective studies were able to control for mortality risks associated with differences in body mass, occupational exposures, smoking (present and past), alcohol use, age, and gender. Both of these studies report a robust and statistically significant association between exposure to particulate matter (measured as PM_{10} or fine particles) and mortality

Additional support for the mortality effects of long-term exposure to particles is provided from a series of cross-sectional studies of the U.S. as a whole (Ozkaynak *et al*., 1987, Evans *et al*., 1984). These cross-sectional studies indicate that county-wide mortality rates across the U.S. are related to annual averages of particulate matter.

MORBIDITY

Many studies have also reported an association between particulate matter and morbidity. For example, daily hospital admissions for respiratory or cardiovascular disease have been linked to daily concentrations of particulate matter in New York City and Buffalo (Thurston, 1992), Ontario, Canada (Burnett, 1995) and Utah Valley (Pope, 1991). Likewise, associations between particulate matter and emergency room visits have been reported (Samet *et al*., 1981, Sunyer *et al*., 1993 and Schwartz *et al*., 1993). Several studies have related particulate matter to increases in exacerbations of asthma (Whittemore *et al*., 1980; Ostro *et al*., 1991; Pope *et al*., 1991).

Among more minor outcomes, particulate matter has been associated with restricted activity days including days spent in bed, days missed from work, and other days when activities are significantly restricted due to illness (Ostro *et al*., 1989), and with lower respiratory symptoms (Ostro *et al*., 1993). Among children, particulates were found to be associated with the presence of chronic cough and bronchitis (Dockery *et al*., 1989). In addition, exposure to particles has been linked to changes in lung function (Dockery *et al*., 1982; Neas *et al*., 1992).

CARCINOGENICITY

Several studies suggest that exposure to air pollution from fossil fuel combustion is associated with increased lung cancer rates independent of smoking and occupation (Cohen and Higgins, 1995; Pope *et al*., 1995; Speizer and Samet, 1994; Dockery *et al*., 1993; Mills *et al*., 1991). For example, Dockery *et al*. (1993) reported that fine particle air pollution (aerodynamic diameter equal to or below 2.5 µm) was positively associated with death from lung cancer and cardiopulmonary disease, but not with death from other causes considered together. The mortality ratio adjusted for smoking and other risk factors was 1.26 (95 percent confidence interval, 1.08 to 1.47), for the most polluted of the cities as compared to the least polluted. Diesel exhaust is a contributor to particulate matter resulting from fossil fuel combustion (Cass

and Gray, 1995). Consequently, increase risk of lung cancer due to ambient diesel exhaust exposure, are supported by epidemiological studies reporting increased lung cancer rates in the general population from fossil fuel combustion.

OTHER SOURCES OF PM

Although this document focuses on diesel exhaust, emissions from sources of PM other than diesel engines (such as agricultural industry and meat cooking) may pose health risks similar to those posed by diesel exhaust, and thus may contribute to similar health problems. It is worthwhile to consider all major sources of PM when contemplating ways to address the health problems associated with PM.

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APPENDIX C

QUANTITATIVE META-ANALYSIS ON THE RELATIONSHIP OF OCCUPATIONAL EXPOSURE TO DIESEL EXHAUST AND LUNG CANCER.

C.1 INTRODUCTION

This appendix reports the results of a meta-analysis on the relationship of occupational exposure to diesel exhaust and lung cancer. A meta-analysis systematically combines the results of previous studies in order to generate a quantitative summary of a particular body of research and to examine the influence of sources of variability among studies (for review see Petitti, 1994). The output from a meta-analysis typically includes a summary estimate of the effect size (relative risk), a measure of the estimate's variability (variance and 95% confidence interval) and a test of homogeneity (the consistency of the estimated effect across studies). Statistical evidence of a lack of homogeneity undermines the validity of a pooled risk estimate, and suggests the need for additional analysis to examine the potential sources of heterogeneity. In this way a meta-analysis not only provides for a quantitative synthesis of research, but also becomes a "study of studies" (Greenland, 1994).

C.2 DATA AND METHODS

C.2.1 IDENTIFICATION AND SELECTION OF STUDIES

Electronic searches were conducted using MEDLINE, TOXLINE, and NIOSHTIC to identify and retrieve all epidemiological studies published from 1975 through 1995 that purported to examine occupational exposure to diesel exhaust as a risk factor for the development of lung cancer. The computerized literature search was supplemented with manual retrieval of additional articles cited in those identified electronically. We initially excluded from consideration studies focusing on mining occupations because of potential confounding from concurrent exposure to other known carcinogens, particularly radon, arsenic and silica, as well as the potential for interaction between cigarette smoking and exposure to these substances in lung cancer induction (Hertz-Picciotto *et al.* 1992, Chau *et al.* 1993, International Agency for Research on Cancer 1988). In the absence of measurements of potential confounders and diesel exhaust in the specific mines under study during the exposure periods of interest, we would not be able to segregate their effects. Since many studies of miners indicate a higher relative risk for lung cancer than those considered in the analysis below, this was a conservative exclusion (See, e.g., Chau *et al.* 1993, Lerchen *et al.* 1987, Damber and Larsson 1987, Burns and Swanson 1991). Forty-seven studies were identified as potentially relevant (Tables C.1 and C.2). Studies were selected for inclusion if they met the following criteria, in addition to having lung cancer as an outcome variable and exposures that included diesel exhaust or an occupation with potential diesel exhaust exposure. First, relative risks (including those estimated by standardized mortality ratios and odds ratios) and their standard errors must be reported or derivable from the information presented. Second, studies must have allowed for an adequate latency period for the development of clinically detectable lung cancer after the onset of exposure, i.e., at least 10 years. Studies lacking enough information to estimate latency from first exposure to follow-up

were also included if the study period appeared to coincide with or follow the target industry's period of dieselization for a sufficient duration. Third, there should be no obvious bias resulting from incomplete ascertainment of cases in follow-up studies, e.g., by excluding cases of lung cancer arising in retirees. Finally, studies must be independent. If more than one study was conducted on the same cohort population, then the study that best met the previous five criteria was selected for inclusion, and the other(s) were excluded as redundant.

Seventeen of the identified studies were excluded for failure to meet one or more of the inclusion criteria (Table C.2). Four studies were excluded due to bias from incomplete ascertainment in cohort studies:; Waller (1981) and Raffle (1957) for excluding post-retirement cases (other than retirements due specifically to lung cancer) in studies of London transport workers, Leupker and Smith (1978) for excluding retirees from a retrospective cohort of teamsters, and Netterström (1988), who included only active bus drivers in a cohort study. Four studies were excluded because of inadequate latency (Decoufle *et al.* 1977, Kaplan 1959; Milne *et al.* 1983; Kaupinnen et al. 1993); one study lacked an appropriate effect measure (Maizlish *et al.* 1988); and seven studies were redundant (Burns and Swanson, 1991; Hall and Wynder, 1984; Boffetta *et al.* 1989; Schenker *et al.* 1984; Damber and Larsson 1985; Siemiatycki 1990; Emmelin *et al.* 1993). In a two-study series on Swedish dock workers, the earlier study by Gustafsson *et al.* (1986) was included in order to include the entire cohort, rather than the more recent nested case-control analysis by Emmelin *et al.* (1993). The report by Williams *et al.* (1977) was excluded because standard errors could not be calculated from the data presented.

C.2.2 DATA EXTRACTION

Meta-analysis requires that estimates of risk and their standard errors be abstracted from the studies under consideration. However, none of the studies meeting the above inclusion criteria reported standard errors. Therefore, we estimated the standard errors based on reported confidence intervals or p-values, using formulae given by Greenland (1987). If an effect was reported as "statistically significant" the p-value was assumed to be 0.049. Likewise, if the estimate was reported with a p-value of <0.01 or < 0.05, then the p-value was assumed to be 0.009 or 0.049, respectively. These are conservative assumptions in that the standard errors derived will be larger than those that would have resulted from assuming a uniform distribution of significant p-values (e.g., between 0.05 and 0.01). For studies that did not report an estimate of the relative risk for lung cancer (Wegman and Peters 1978; Edling *et al.* 1987), risk estimates and approximate 95% confidence intervals were calculated using Woolf's and Byar's formulae (Checkoway *et al.* 1989, Breslow and Day 1987). From these confidence intervals, the standard errors were estimated, again using formulae given by Greenland (1987). All risk estimates and standard errors were transformed to the natural logarithm scale prior to analysis. Using the natural logarithm of the relative risk creates an equivalence of distance above and below the null value (e.g., the "distance" between a relative risk of 2 and the null value of 1 is the same as the distance between a relative risk of 0.5 and 1). The logarithmic transformation tends to make the probability distributions of the relative risks more nearly normal, avoiding "ratio bias" and leading to greater mathematical tractability than would the use of untransformed ratios (Berlin *et al.* 1993; Moolgavkar and Venzon 1987).

Many studies reported estimates of relative risk associated with: (1) several levels of exposure or different exposure categories, such as duration of employment or cumulative diesel exhaust exposure; (2) job or industry subgroups, such as truck drivers and bus drivers; and (3) adjustment for potential confounders, such as smoking or asbestos exposure. In such cases, all relevant estimates of relative risk were extracted. The primary analysis, however, was conducted using data selected in the following manner. If a study reported effects for more than one level of exposure, the effect reported for the highest level of exposure was chosen. If stratification by years of exposure or employment was reported, the effect measure corresponding to the stratum with the longest duration of exposure was selected, in order to provide the greatest potential sensitivity in calculating pooled estimates of effect. If, however, multiple strata with 20 or more years of exposure were available, a pooled effect measure was calculated by the general variance-based method (Greenland 1987). The most diesel-specific occupation or exposure group was selected. For example, if a study presented estimates for both a broad occupational category, such as all professional drivers (truck, taxi and bus), and a more specific occupation or subset, such as truck drivers only, then the truck driver risk estimate was extracted for analysis. Where a given study presented crude and adjusted estimates of relative risk, the adjusted estimates were used. Several risk estimates were extracted from six studies reporting results for multiple diesel-related occupational subgroups (Benhamou *et al.* 1988; Boffetta *et al.* 1988; Hayes *et al.* 1989; Menck and Henderson 1976; Steenland *et al.* 1990; and Swanson *et al.* 1993). These risk estimates were associated with mutually exclusive occupational subpopulations, so that there was no double-counting of person-time experience among the lung cancer cases. Gustavsson *et al.* (1990) included estimates based on a retrospective cohort and a nested case-control study of the same population. Since the cohort-based estimates included mesotheliomas as well as lung cancer, while the case-control study did not, the meta-analysis only used estimates based on the latter approach. The meta-analysis included a total of 39 risk estimates from 30 studies (Table C.1).

C.2.3 ANALYSIS

There are two general statistical approaches used in meta-analyses to derive summary estimates of risk. If all studies being combined are thought to estimate the same underlying effect size or risk, then a fixed-effects model is appropriate. Under this model, the variation in risk estimates among multiple studies is assumed to be solely due to random variation and the risks are said to be homogeneous across studies (Petitti, 1994). Conversely, random-effects models are predicated on the assumption that there is not a single parameter that represents the "true" risk in all study populations, but rather that there is a range of effect sizes, with a given central value and variance. In random-effects models, the results across studies are considered a priori to be heterogeneous and the risk estimates in the individual studies are treated as a random sample from the universe of risk estimates that have been, and could be, generated from all potential study populations (DerSimonian and Laird, 1986; Greenland and Salvan 1990).

Given the multifactorial etiology of lung cancer, the variability of exposures across studies, and the range of confounders and effect modifiers across diesel exhaust-exposed study populations, it would be unrealistic to assume that the underlying risks estimated by each of the studies included in this meta-analysis have a single common value. Thus, in addition to the general variance-based method for fixed effects described by Greenland (1987), the random-effects model

proposed by DerSimonian and Laird, which allows for heterogeneity in risk estimates across studies, was also used for this analysis (DerSimonian and Laird 1986). Under this model, a pooled estimate of relative risk (pooled RR) is calculated as a weighted average of the risks reported in each study. Each study is weighted by a factor equal to the inverse of the variance of the "true" underlying effect size (estimated by the among-study variance [τ^2]) added to its own within-study variance [σ^2]. This model is a modification of the inverse variance-weighted method used in fixed-effects models and allows for homogeneity as a special case. Specifically, if the among-study variance is found to be less than expected under the assumption of homogeneity (i.e., $p > 0.50$), then $\tau^2 = 0$ and the model reduces to a fixed-effects model (Berlin *et al.* 1989). We evaluated the significance of the among-study variance based on the value of the Q-statistic, which has a chi-square distribution with degrees of freedom equal to one less than the number of studies pooled in the analysis. A low p-value for this statistic is indicative of heterogeneity (DerSimonian and Laird 1986).

Because significant among-study variance was detected (see Table C.3), we evaluated the potential sources of heterogeneity by subset analysis. Categorical variables were created to characterize each study's design (cohort or case-control), target population (general or industry-specific), occupational subtype (trucking, railroad, bus drivers, dock workers, diesel mechanics or garage workers, professional drivers or general transportation not otherwise specified, or, in some studies, diesel-exposed versus non-diesel-exposed occupations), source of control or reference population (hospital-based, other cancer cases, general population, internal or other occupationally active population, regional or state rates, or national rates), latency (greater than 10 years or undefined), duration of exposure (with intervals of 10 and 20 years), method of identifying cases (tumor registry, hospital-based, census, death certificate, or multiple sources), method of ascertaining occupation (interview/questionnaire, census, job or union records), year of publication, location (North America or Europe), and which covariates were controlled for in the analysis (age, smoking, and asbestos exposure), and the presence of a clear healthy worker effect (as manifested by lower than expected all-cause mortality in the occupational population under study). These study characteristics were then used in the subset analyses to explore sources of heterogeneity.

For subset analysis, we repeatedly performed the analyses, re-grouping the data by these study characteristics, calculating subgroup-specific pooled RRs and comparing these pooled RRs across groups. A factor was considered to be an important source of heterogeneity if stratification on that factor markedly affected the heterogeneity of stratum-specific estimates of effect.

In occupational and environmental epidemiology, detailed exposure measurements for the subjects under study are the exception rather than the rule. Therefore, in these types of studies, indirect measures or surrogates of exposure, such as job titles or duration of employment, are often used. The absence of direct exposure data does not invalidate the analysis; rather, combining studies together that have different, indirect measures of exposure can contribute to heterogeneity (Blair et al. 1995). Because this meta-analysis was based on occupational epidemiological studies involving diesel exhaust exposures in which there were no concurrent industrial hygiene measurements, OEHHA staff relied on surrogate measures of exposure as

assessed primarily by job titles and duration of employment. Recognizing that this would likely be a significant source of heterogeneity, OEHHA staff stratified the data based on occupational categories in one set of analyses (e.g., truck drivers, railroad workers), in order to examine the effect that this would have on the heterogeneity among the pooled estimates in these occupational subsets.

Sensitivity analyses were conducted to evaluate the robustness of our results with regard to inclusion criteria used and assumptions made during the study selection and data extraction phases. By selecting different sets of studies used in the analysis, the influence of several criteria were evaluated with respect to their effect on the total and subgroup-specific pooled RRs, specifically: (1) exclusion of studies in which exposure to diesel exhaust appeared to be less certain than in the remainder of the studies, and (2) substitution of excluded "redundant" studies for those that had been included. Influence analyses that involved repetition of the analysis while dropping one study at a time were also conducted to examine whether any studies disproportionately influenced the results.

Publication bias, or the increased likelihood or preference for the publication of statistically significant results compared to nonsignificant or null results, may potentially distort a pooled risk estimate. Publication bias is generally attributed to journal editorial policies that prefer "positive" results, so that small, statistically insignificant studies are less likely to be published than large, statistically insignificant studies (Greenland, 1994). One way to assess graphically whether publication bias is likely to have affected the results of a meta-analysis is to construct funnel plots of the logarithms of the relative risk (log RRs) versus sample size. If there is no publication bias, the plot should resemble an inverted funnel with the apex located approximately over the mean log RR. In order to examine whether the data set used in this meta-analysis could have been affected by publication bias, we created a funnel plot of the inverse of the study standard error against the logarithm of the estimate of the effect size. In addition, we undertook a subset analysis examining the effect of stratifying studies based on the size of their standard errors, which is effectively a sensitivity analysis based on study sample size.

Microsoft Excel Version 5.0 (Microsoft Corporation, Redmond, WA) and PC-SAS Version 6.12 (SAS Institute, Inc., Cary, NC) were used to conduct the statistical analysis.

C.3 RESULTS

Thirty studies, contributing a total of 39 effect estimates to this meta-analysis, are summarized in Table C-1. The estimates of relative risk and 95% confidence intervals (C.I.) for each study estimate, including the six studies with multiple-occupation risk estimates, are presented. All but 5 of the 30 studies reported at least one positive association between diesel exhaust-related occupations or exposure and lung cancer (relative risk estimate greater than 1.0), although many were not statistically significant at $\alpha = 0.05$. As listed in Table C.3, the pooled RR for lung cancer from all 39 risk estimates combined was 1.04 (95% C.I. = 1.02-1.06) under the fixed-effects model and 1.33 (95% C.I. = 1.21-1.46) with the random-effects model. Significant evidence of heterogeneity was found (DerSimonian and Laird Q-statistic = 214.59, 38 d.f., [$p<0.001$]).

Subset analyses identified several major potential sources of heterogeneity. Table C.3 presents several subgroup-specific pooled RRs under both models. A modestly higher pooled estimate of risk was derived for the subset of case-control studies (pooled RR = 1.44, 95% C.I. = 1.33-1.56, random effects model). In contrast to the overall risk estimate, the case-control subgroup showed little evidence of heterogeneity, giving equivalent estimates under both models. However, the pooled risk estimate for the cohort study subset still displayed evidence of significant heterogeneity (Q statistic = 77.66, 17 d.f., $p<0.001$). Subsetting the cohort studies into those with and those without an obvious healthy worker effect (HWE - based on lower than expected all-cause mortality) markedly reduced the degree of heterogeneity in the group without the HWE (Q-statistic = 11.19, 9 d.f., $p = 0.26$), and produced an increase in the magnitude of the pooled relative risk estimate (RR = 1.52, 95% C.I. = 1.36-1.71, random-effects model). Subanalysis of the case-control studies by the type and source of control or comparison population gave pooled RR estimates ranging from a low of 1.05 (general population controls, , 95% C.I. = 0.73-1.50) to 1.85 (cancer controls, 95% C.I. = 1.11-3.09), though the latter pooled estimate showed significant heterogeneity (Q-statistic = 8.21, 3 d.f., $p = 0.04$). Pooled estimates for cohort studies continued to lack homogeneity after subsetting by source of the reference population, though heterogeneity was substantially reduced in the subgroup of studies in which the reference population was either internal or occupationally active. Moreover, there was a trend of decreasing estimated pooled risk corresponding to a reference population that resembled more the general population than the study population (i.e., the estimated risks were higher when the reference population included an internal or other occupationally active cohort, decreasing when regional, state or national comparison rates were used). Not surprisingly, almost all of the cohort studies comprising the group with a clear HWE used national rates for comparison.

Whether the studies adjusted for smoking appeared to be a major source of heterogeneity. The 12 studies (20 risk estimates) that adjusted for smoking showed little evidence of heterogeneity (Q statistic = 20.24, 19 d.f., $p = 0.38$), and resulted in virtually identical pooled RR estimates of 1.44-(95% C.I. = 1.32-1.56) and -1.43 (95% C.I. = 1.31-1.57) for the fixed- and random-effects models, respectively. By comparison, the studies that did not adjust for smoking (19 risk estimates) retained a large amount of heterogeneity (Q statistic = 129.10, 18 d.f., $p<0.001$), and produced lower pooled risk estimates under both fixed- and random-effects models.

Subset analyses by specific occupations demonstrated pooled RR estimates with little evidence of heterogeneity for truck drivers (random-effects pooled RR = 1.47, 95% C.I. = 1.33-1.63), general transportation and professional drivers (random-effects model pooled RR = 1.45, 95% C.I. = 1.31-1.60), or the grouped diesel exhaust-exposed populations (random-effects pooled RR = 0.97 (95% C.I. = 0.97-1.00). Although the railroad industry also gave higher estimates than the all-studies risk estimate, a large amount of heterogeneity remained within this subset (Q statistic = 30.90, 5 d.f., $p<0.001$). Figures C.1 to C.4 illustrate both the pooled RR estimates and the individual study risk estimates for two occupational categories, as well as for studies that adjusted or did not adjust for smoking.

Stratifying the data on other study characteristics, including region, source population, exposure duration, latency, and method of job ascertainment, yielded point estimates of pooled RRs ranging from 1.00 to 1.70, almost all of which were statistically significant. However, most of

these subgroup analyses indicated the presence of heterogeneity. Moreover, in a majority of the studies, neither the latencies nor the duration of the study populations' employment (the most common surrogate for exposure in these reports) were clearly evident.

Additional stratification was conducted by two variables at a time (Table C.4): smoking and occupation; smoking and exposure duration; and occupation and duration. As expected, pooled RR estimates by occupation in smoking-adjusted studies showed little evidence of heterogeneity under both models for virtually all occupational subgroups. In the studies not adjusted for smoking, heterogeneity was substantially reduced after stratification, but remained especially prominent among studies of railroad workers (Q statistic = 21.52, 2 d.f., $p<0.001$). Studies of truck drivers that did not adjust for smoking still produced a random-effects pooled RR of 1.46 (95% C.I. = 1.30-1.64) with little evidence of heterogeneity. Stratification by adjustment for smoking and exposure duration (years of employment in diesel-related occupations) showed some evidence of increasing risk with increasing duration of employment in smoking-adjusted studies, with point estimates of 1.39, 1.64, and 1.64 for durations of exposure of < 10 yr, >10 yr, and >20 yr, respectively.

Several occupations were analyzed by duration of exposure, though this resulted in small numbers of studies in each stratum (Table C.4). Pooled estimates for truck drivers with either greater or less than 20 years of exposure as measured by duration of employment were 2.41 (95% C.I. = 1.53-3.81) and 1.51 (95% C.I. = 1.18-1.95), respectively. In the three studies of railroad workers with sufficient information to identify exposure duration exceeding 10 years, the pooled relative risk estimate was 1.76 (95% C.I. = 1.40-2.21).

Several sensitivity analyses did not substantially alter the results. Including the studies that were initially excluded because they were redundant with other studies, and excluding the studies originally included, did not markedly change the pooled RR estimates or those derived for the occupation-specific subgroups (Table C-5). Similarly, the addition of the four studies excluded for lack of adequate latency (Decoufle *et al.* 1977, Milne *et al.* 1983; Kaplan, 1959; Kaupinnen 1993) did not affect the overall pooled RR estimates.

By selectively excluding one study at a time, the influence analysis did not reveal any as particularly influential to the overall random-effects estimate, though exclusion of the Magnani study increased the pooled fixed-effects estimate from 1.04 (95% C.I. - 1.02 - 1.06) to 1.22 (95% C.I. =1.18 - 1.27). However, regardless of the specific study removed or model used, substantial heterogeneity remained ($p<0.001$ for all Q statistics). A similar analysis was then conducted within occupational subgroups to identify potentially influential studies in the more homogeneous exposure groups that included five or more studies, including truckers, railroad workers, diesel mechanics, professional drivers and general transportation operatives. As presented in Table C.6, by subsetting on occupation, several studies appeared to influence the occupation-specific pooled RR estimates. Estimates for the truck driver subgroup ranged from a low of 1.44 (95% C.I. = 1.30-1.59) after the removal of Swanson *et al.* (1993) to a high of 1.56 (95% C.I. = 1.37-1.77) excluding the estimate from Ahlberg *et al.* (1981). As in the initial subgroup analysis, pooled RR estimates for railroad workers continued to display the greatest heterogeneity and differences between the two models, with one exception. After excluding the

risk estimate from the study of Nokso-Koivisto and Pukkala (1994), the pooled RR increased to 1.50 (95% C.I. = 1.31-1.71) under the fixed-effects model with little evidence of heterogeneity, while the random-effects model resulted in a pooled RR of 1.53 (95% C.I. = 1.31-1.80). For the more general occupational categories of transportation and professional drivers, pooled RR estimates ranged from 1.40 to 1.47 under both fixed- and random-effects models after excluding Pfluger and Minder (1994) and Buiatti *et al.* (1985), respectively, with all pooled estimates demonstrating a lack of significant heterogeneity.

With no direct measurement of diesel exhaust exposure in the populations studied, assessment of exposure is clearly problematic. However, in several studies it was not possible to distinguish diesel from internal combustion engine exhaust exposures (Benhamou *et al.* 1988, Buiatti *et al.* 1985, Wegman and Peters 1978, Hayes et al 1989, Bender *et al.* 1989, and Balarajan and McDowall 1988). Thus, one of the sensitivity analyses involved re-running some of the subgroup calculations, omitting these studies. Interestingly, this had little effect on the overall pooled estimates or on those derived from specific occupational subgroups. For example, the point estimates for case-control studies increased slightly from 1.44 to 1.46; for smoking-adjusted studies from 1.43 to 1.47, and for smoking-adjusted studies in which the participants had had 10 or more years of exposure from 1.64 to 1.68. As expected, the confidence intervals were slightly wider with fewer studies contributing to the pooled estimates. Similarly, for occupational subgroups affected by these exclusions, pooled RR estimates changed little. For instance, the random-effects pooled estimates for truck drivers (minus two estimates) went from 1.47 to 1.50, for mechanics (minus 2 estimates) from 1.35 to 1.41, and for professional drivers and transportation operatives (minus four estimates) from 1.47 to 1.48. No studies of railroad workers were excluded in this sensitivity analysis.

The funnel plot (Figure C.5) revealed no systematic relationship between study size and magnitude of risk indicative of publication bias. The lower left portion of the plot, which represents small, null studies, is modestly less dense than the lower right. However, in both sets of study designs, estimates from the smaller studies spanned the range of relative risks. In a sensitivity analysis in which the studies were divided into tertiles corresponding to the size of their standard errors, the pooled RR estimates under the random-effects model for the studies with the smallest (range = 0.013-0.145), middle (range = 0.148-0.272), and greatest (range = 0.331-0.630) standard errors were 1.20 (95% C.I. = 1.07-1.35), 1.42 (95% C.I. = 1.26-1.60), and 1.72 (95% C.I. = 1.34-2.21), respectively. There was substantial evidence of heterogeneity in the former tertile (Q-statistic = 133.82, 12 d.f., $p<0.001$), and little evidence of heterogeneity in the latter two tertiles.

C.4 DISCUSSION

The results of this meta-analysis indicate a consistent positive association between occupations involving diesel exhaust exposure and the development of lung cancer. Although substantial heterogeneity existed in the initial pooled analysis, stratification on several factors identified a relationship that persisted throughout various influence and sensitivity analyses. The subset analysis demonstrated that major sources of heterogeneity involved several aspects of study design, which included controlling for smoking, exposure assessment as assigned through

occupational categories, as well as selection bias, as represented by the presence of the healthy worker effect.

As is often the case, subgroup analysis by study design produced different pooled estimates for case-control versus cohort studies. The pooled RR estimates in the case-control studies was somewhat greater than that observed for the overall pooled estimates; however, with little evidence of heterogeneity. In contrast, the cohort study subgroup retained substantial heterogeneity. Stratifying the cohort studies by the presence or absence of a general healthy worker effect (HWE) produced increased pooled risk estimates in both fixed- and random-effects models, with less evidence of heterogeneity in the subgroup without a clear HWE, while the opposite occurred for those studies whose results were characterized by the presence of a HWE. The HWE is a manifestation of selection bias related to hiring and retention of workers who are typically healthier than the general population, resulting in spuriously lower risk estimates for a variety of illnesses, including those potentially related to occupational exposures. Even after subsetting the cohort studies into those with and without an HWE, the results reported for the former subgroup retained considerable residual heterogeneity, indicating that the pooled risk estimates for studies with a HWE are of doubtful statistical validity.

By stratifying the 39 risk estimates on whether the studies adjusted for cigarette smoking, the effect of failure to control for this exposure on the pooled estimate became readily apparent. Not only did the positive association between diesel-exhaust exposure and lung cancer persist, but the pooled risk estimate increased to 1.43 (95% C.I. = 1.31-1.57) with little evidence of heterogeneity among the 20 smoking-controlled risk estimates (from 12 studies). The influence of the lack of smoking adjustment over the other study parameters investigated was illustrated in the subgroup analysis by study design. Whether the lower pooled RR estimate and substantial heterogeneity obtained from the cohort subanalysis was due to factors other than adjustment for smoking remains uncertain, as only one of sixteen cohort studies controlled for this confounder (accounting for 3 of 18 cohort-derived estimates) while most case-control studies did (11 of 14 studies, accounting for 17 of the 20 case-control risk estimates).

Statistically significant pooled estimates of elevated risk were identified in most of the occupational subgroup analyses, several of which had sufficient numbers of studies to undertake additional stratification for smoking. The occupational subgroups involving trucking (pooled RR = 1.47, 95% C.I. = 1.33-1.63) and professional drivers and general transportation operatives (random-effects pooled RR = 1.45, 95% C.I. = 1.31-1.60) gave risk estimates greater than the overall pooled risk estimates, and showed little evidence of heterogeneity. The DE grouped studies produced a lower pooled estimate (RR = 0.97, C.I., = 0.95-1.00), which also showed little evidence of heterogeneity. In contrast, estimates for the railroad industry demonstrated considerable heterogeneity (Q statistic = 30.90, 5 d.f., $p<0.001$).

Stratifying the occupational subgroup analysis by adjustment for smoking produced a large impact on the pooled risk estimates, with all smoking-adjusted subgroup estimates displaying a lack of heterogeneity and leading to increased risk estimates in all but two of the occupational categories. Analyses of smoking-adjusted studies of truck drivers, railroad workers, heavy equipment operators and dock workers, and grouped diesel-exposed workers all produced pooled

estimates higher than those produced in the subgroup analyses that were not stratified on control for smoking, while this was not observed for mechanics or for professional drivers and transportation operatives. The pooled estimates for the heavy equipment operators and dock workers and for the railroad industry studies adjusting for smoking displayed the most dramatic changes relative to the occupational analysis without smoking stratification. Among the former subgroup, the pooled risk estimate changed from 1.28 (random-effects model, 95% C.I. = 0.99-1.66) to 2.43 (95% C.I. = 1.21-4.88) Among the railroad industry studies, the pooled risk estimate also increased substantially (from 1.45 [95% C.I. = 1.08-1.93] to 1.68 [95% C.I. = 1.28-2.19]). In these two groups of studies, the pooled smoking-adjusted estimates showed little evidence of heterogeneity, though these estimates were based on two studies in the former instance and three in the latter. However, the other two heavy equipment operator and dock worker studies and the other three railroad industry studies that were not adjusted for smoking still retained evidence of substantial heterogeneity (Q-statistics = 2.933, 1 d.f., $p = 0.09$, and 21.517, 2 d.f., $p<0.001$, respectively).

Although no single study was found to significantly influence the overall random-effects pooled risk estimate, within the occupational subgroups the elimination of individual studies appeared to alter the size or heterogeneity of the pooled risk estimates (Table C.6). This was particularly true in the railroad industry subanalysis. As previously discussed, the pooled risk estimate for this occupational subgroup (n = 6 studies), although elevated, displayed a significant lack of homogeneity. Removing the estimate obtained from the retrospective cohort described by Nokso-Koivisto and Pukkala (1994), one of three railroad studies that did not control for smoking, resulted in pooled RRs of 1.50 and 1.53 (fixed-effects and random-effects models, respectively), with little evidence of heterogeneity (Q statistic = 4.73, $p = 0.331$). None of the other railroad industry studies had such a profound effect on the pooled estimates.

It is not clear why the risk estimates from the study by Nokso-Koivisto and Pukkala (1994) differ so markedly from those reported in the other investigations of railroad workers. This was the only European railroad study, and included only locomotive engineers, while the American and Canadian studies included a variety of railroad-related occupations. Spanning approximately four decades (1953-1991), the study encompassed the transition from steam to diesel engines. The earlier cases were unlikely to have experienced any diesel exposure, though they were undoubtedly exposed to asbestos during steam engine maintenance. Also, along with Howe *et al.* (1983), a study involving Canadian railroad workers, the lung cancer mortality experience of the Finnish cohort was compared with national rates, which would tend to give lower risk estimates than those using other reference populations. Finally, though the smoking prevalence of railroad workers in Finland in 1976 was reported to be comparable to that of the Finnish population as a whole, the investigators indicated that during the steam engine era, which lasted into the 1960s, locomotive cabin conditions were not conducive to smoking on the job. The implication of this observation is that during the early years covered by this investigation, the study subjects may not have smoked as much as the general population, which in turn would lead to lower lung cancer rates in subsequent years.

Subgroup analysis based on duration of exposure, measured as years of employment, was hampered by the absence of duration-specific risk estimates in more than one-half the studies (22 estimates from 19 studies). Among the studies with an unclear exposure duration, and studies

with ≥ 10 yr or ≥ 20 yr duration of exposure, there remained substantial heterogeneity and no clear evidence of an exposure-response trend. Within two of the occupational subgroups with sufficient numbers to identify duration-specific estimates, there was modest evidence of such a trend. Pooled RRs for truck drivers were 2.41 (95% C.I. = 1.53-3.81) for those with ≥ 20 yr and 1.51 (95% C.I. = 1.18-1.95) with less than 20 yr employment, while for those with an unclear exposure duration, the pooled RR was 1.41 (95% C.I. = 1.27-1.58). These were based on few studies (2, 2, and 5, respectively). By stratifying the 20 smoking-adjusted risk estimates (from 12 studies) on duration of exposure, evidence suggestive of an exposure-response relationship was observed in the pooled risk estimates, although here again the lack of exposure duration-specific estimates in five of these studies substantially reduced the degrees of freedom in the pooled estimates. Under the random-effects model, the pooled risk estimate of 1.39 in the stratum representing the shortest potential exposure (< 10 years) increased to 1.64 for exposures of at least 10 or 20 yr. In contrast to the duration-only subgroup analysis, all RRs from these smoking-adjusted duration strata lacked significant evidence of heterogeneity.

Although diesel engines have been utilized for transportation since the 1930s, their widespread use has occurred primarily since the 1950s by varying degrees within specific industries and countries. For example, most U.S. trucks, except in the Western states, did not use diesel engines until the late 1950s or early 1960s, with many smaller short-haul trucks still powered by gasoline engines. With the increase in the utilization of diesel engines, temporal and geographic patterns of occupational exposure to diesel-based versus other petroleum-based combustion products have changed. Uncertainties in the timing of dieselization by specific industry may result in exposure misclassification, possibly overestimating the exposure to diesel exhaust. In the case of trucking, bus company workers and other professional drivers, some of the person-time experience underlying the elevated risk estimates probably corresponds to exposure to internal combustion engine exhaust. An evaluation by the International Agency for Research on Cancer (IARC, 1989) found gasoline combustion exhaust to be relatively less carcinogenic than diesel exhaust. If this is true, misclassification of exposure to gasoline exhaust as diesel exposure could bias the results towards the null hypothesis of no association between diesel exhaust exposure and lung cancer in these populations.

Having an internal or other occupationally active reference group was identified as another factor contributing to heterogeneity. Among cohort studies, those with internal controls showed higher pooled risk estimates (under both models with little evidence of heterogeneity), than those using regional/state, or national comparison rates with the latter groups retaining ample heterogeneity (Table C.3). Among the case-control studies, the use of an internal or other occupationally active reference group produced one of the higher pooled risk estimates, though this issue would be expected to be less influential in the case-control than in the cohort studies, since virtually all of the former category collected data on and controlled for the potential confounding influence of cigarette smoking. Among all studies that used internal controls (n = 10 estimates from 7 studies), there was no evidence of heterogeneity, and the estimate of the pooled RR was 1.51 (95% C.I. = 1.39 - 1.64), while among those that used other genres of reference populations, there was still substantial heterogeneity (Q-statistic = 121.34, 28 d.f., $p<0.001$), with a pooled random effects RR of 1.23 (95% C.I. = 1.12-1.36).

A meta-analysis involves an examination of study characteristics rather than individual-level data. In this meta-analysis we did not construct a formal index to represent study quality. Interestingly, in this analysis, factors consistent with higher study quality contributed to higher pooled estimates of risk, including: (1) adjustment for smoking, and (2) having a lower likelihood of selection bias manifested by both the absence of a healthy worker effect and the use of an internal or occupationally active comparison group. These quality indicators clearly overlap, however. For example, the use of internal or other occupationally active controls in cohort studies tends to decrease the selection bias underlying the healthy worker effect and to diminish potential effects of confounding by cigarette smoking by effectuating similar distributions of smoking in the study and comparison populations.

Concern about publication bias is more acute in random-effects than fixed-effects models, as the former tend to weight studies more evenly. By adding the among-study variances to the within-study variances to derive weights for estimating the pooled RRs, estimates derived from random-effects models may be more sensitive to the effects of large risk estimates derived from small studies. However, many of the studies in this meta-analysis focused on several potential chemical exposures, adverse health outcomes, or occupations with variable diesel-exhaust exposure patterns, so that the diesel-exhaust-to-lung cancer relationship represented only one aspect of these studies. Furthermore, a plot of the RRs versus sample size for each study design revealed no systematic relationship between study variability and magnitude of risk, though there is a lower density of studies in the lower left portion of Figure C-5, indicating fewer small, statistically insignificant studies. The sensitivity analysis, involving tertiles of studies divided on the basis of the size of their standard errors, suggests that the larger studies showed lower pooled estimates of relative risk. While this result suggests the presence of some potential publication bias, it should be noted that the studies in the tertile with the smallest standard errors were almost exclusively cohort studies that did not adjust for smoking and which also had a clear HWE, suggesting that other significant biases are likely to have played a role in creating an appearance of publication bias. Furthermore, it should be noted that this tertile also manifested serious heterogeneity, indicating that the pooled estimate for this stratum is of dubious validity, even if it is statistically significant. Although publication bias cannot be ruled out, the inclusion of numerous studies of varying sample sizes and statistically insignificant findings makes it unlikely that the results can be completely explained by publication bias.

This meta-analysis provides evidence consistent with the hypothesis that exposure to diesel exhaust is associated with an increased risk of lung cancer. The pooled estimates clearly reflect the existence of a positive relationship between diesel exhaust and lung cancer in a variety of diesel-exposed occupations, which is supported when the most important potential confounder, cigarette smoking, is measured and controlled. There is suggestive evidence of an exposure-response relationship in the smoking-adjusted studies as well. Many of the subset analyses indicated the presence of substantial heterogeneity among the pooled estimates. Much of the heterogeneity observed, however, is due to the presence or absence of adjustment for smoking in the individual study risk estimates, to occupation-specific influences on exposure, to potential selection biases, and other aspects of study design.

Table C-1. Studies Included in Meta-analysis of Diesel Exhaust Exposure and Lung Cancer

Study (year)	Design	Location	Occupation or Exposure Group	Smoking Adjusted	RR	C.I.
Ahlberg *et al*. (1981)	Cohort	Europe	Truck drivers	no	1.33	1.13-1.56
Balarajan & McDowall (1988)	Cohort	Europe	Truck drivers	no	1.59	1.00-2.53[a]
Bender *et al*. (1989)	Cohort	North America	Highway maintenance	no	0.69	0.52-0.90
Benhamou *et al*. (1988)	Case-control	Europe	Professional drivers	yes	1.42	1.07-1.89
Buiatti *et al*. (1985)	Case-control	Europe	Transportation general	yes	1.1	0.7-1.6
Benhamou *et al*. (1988)	Case-control	Europe	Mechanics	yes	1.06	0.73-1.54
Boffetta *et al*. (1988)	Cohort	North America	Truck drivers	yes	1.24	0.93-1.66
	Cohort	North America	Railroad workers	yes	1.59	0.94-2.69
	Cohort	North America	Heavy equipment operators	yes	2.60	1.12-6.06
Boffetta *et al*. (1990)	Case-control	North America	Probable DE $\geq$ 30 yr	yes	1.49	0.72-3.11
Coggon *et al*. (1984)	Case-control	Europe	Diesel exhaust exposed group	no	1.1	0.7-1.8
Damber & Larsson (1987)	Case-control	Europe	Professional drivers	yes	1.2	0.6-2.2
Edling *et al*. (1987)	Cohort	Europe	Bus drivers	no	0.69[b]	0.2-1.6[b]
Garshick *et al*. (1987)	Case-control	North America	Railroad workers $\geq$ 20 yrs[c]	yes	1.55	1.09-2.21
Garshick *et al*. (1988)	Cohort	North America	Railroad workers $\geq$ 15 yrs[c]	no	1.82	1.30-2.55
Guberan *et al*. (1992)	Cohort	Europe	Professional drivers	no	1.50	1.23-1.81[e]
Gustafsson *et al*. (1986)	Cohort	Europe	Dock workers	no	1.32	1.05-1.66
Gustvasson *et al*. (1990)	Nested case-control	Europe	Bus garage workers > 20 yr[d]	no	1.49[d]	1.25-1.77[d]
Hansen (1993)	Cohort	Europe	Truck drivers	no	1.6	1.26-2.0
Hayes *et al*. (1989)	Case-control	North America	Truck drivers $\geq$ 10 yr	yes	1.5	1.1-2.0
	Case-control	North America	Bus drivers $\geq$ 10 yr	yes	1.7	0.8-3.4
	Case-control	North America	Mechanic (excl auto) $\geq$ 10 yr	yes	2.1	0.9-5.2
	Case-control	North America	Heavy equipment operators $\geq$ 10 yr	yes	2.1	0.6-7.1
Howe *et al*. (1983)	Cohort	North America	Railroad workers probably exposed	no	1.35	1.13-1.61[a]

[a] Calculated from p-value.
[b] Calculated from data presented in publication.
[c] Risk estimates excluding shop workers.
[d] Pooled risk estimates from two racial or duration categories.
[e] 90% confidence intervals originally presented within study.

DE = diesel exhaust
RR = risk ratio
C.I.= 95% confidence interval.

Table C-1. Studies Included in Meta-analysis of Diesel Exhaust Exposure and Lung Cancer (continued)

Study (year)	Design	Location	Occupation or Exposure Group	Smoking Adjusted	RR	C.I.
Lerchen *et al*. (1987)	Case-control	North America	Diesel exhaust grouped	yes	0.6	0.2-1.6
Magnani *et al*. (1988)	Death certificate study	Europe	Diesel exhaust grouped	no	0.97	0.95-1.00
Menck & Henderson (1976)	Cohort	North America	Truck drivers	no	1.65	1.13-2.40[a]
	Cohort	North America	Mechanic (excl auto)	no	3.32	1.35-8.18[a]
Nokso-Koivisto & Pukkala(1994)	Cohort	Europe	Railroad workers	no	0.90[d]	0.79-1.04[d]
Pfluger & Minder (1994)	Case-control	Europe	Professional drivers	yes	1.48	1.30-1.68
Rafnsson & Gunnarsdottir (1991)	Cohort	Europe	Truck drivers $\geq$ 30 yr	no	2.32	0.85-5.04
Rushton *et al*. (1983)	Cohort	Europe	Bus garage workers/mechanics	no	1.01	0.82-1.22
Siemiatycki *et al*. (1988)	Case-control	North America	Diesel exhaust grouped	yes	1.1	0.8-1.5[e]
Steenland *et al*. (1990)	Case-control	North America	Truck drivers $\geq$ 18 yr	yes	1.55	0.97-2.47
	Case-control	North America	Truck mechanic $\geq$ 18 yr	yes	1.50	0.59-3.40
Swanson *et al*. (1993)	Case-control	North America	Heavy truck drivers $\geq$ 20 yr	yes	2.44[d]	1.43-4.16[d]
	Case-control	North America	Railroad workers $\geq$ 10 yr	yes	2.46[d]	1.24-4.89[a]
Wegman & Peters (1978)	Case-control	North America	Transportation equipment operators	no	2.39[b]	0.70-8.05[b]
Wong *et al*. (1985)	Cohort	North America	Heavy equipment operators $\geq$ 20 yr	no	1.07	1.00-1.15[a]

[a] Calculated from p-value.
[b] Calculated from data presented in publication.
[c] Risk estimates excluding shop workers.
[d] Pooled risk estimates from two racial or duration categories.
[e] 90% confidence intervals originally presented within study.

DE = diesel exhaust
RR = risk ratio
C.I.= 95% confidence interval.

Table C-2. Studies Excluded from Meta-analysis of Diesel Exhaust Exposure and Lung Cancer

Study (year)	Reason for Exclusion	Occupation or Exposure Group
Boffetta *et al.* (1989)	Redundant study/duplicate report	Grouped diesel exposure
Burns & Swanson (1991)	Redundant study	General population
Damber & Larsson (1985)	Redundant study	Professional drivers
Decoufle *et al.* (1977)	Inadequate latency	Professional Drivers
Emmelin *et al.* (1993)	Redundant study	Dock workers
Hall & Wynder (1984)	Redundant study	Grouped diesel exposure
Kaplan (1959)	Inadequate latency	Railroad workers
Kauppinen et al. (1993)	Inadequate Latency	Woodworkers
Luepker & Smith (1978)	Excluded retirees	Truck drivers
Netterström (1988)	Excluded retirees	Bus Driver
Maizlish *et al.* (1988)	Inappropriate effect measure	Highway maintenance
Milne *et al.* (1983)	Inadequate latency	Truck and bus drivers
Raffle (1957)	Excluded retirees	Bus and trolley
Schenker *et al.* (1984)	Redundant study	Railroad workers
Siemiatycki *et al.* (1990)	Redundant study	Grouped diesel exposure
Waller (1981)	Excluded retirees	Bus company
Williams *et al.* (1977)	Inadequate data presentation	Railroad workers
Williams *et al.* (1977)	Inadequate data presentation	Truck drivers

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Table C.3 Analysis of Studies of Diesel Exhaust Exposure and Lung Cancer: Results of Analysis Using Fixed- and Random-Effects Models

GROUP (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
ALL STUDIES (n=39/30)	0.011	1.04	1.02-1.06	214.593	38	0.000	0.043	1.33	1.21-1.46
BY STUDY DESIGN									
Cohort (n=18/15)	0.024	1.15	1.10-1.21	77.655	17	0.000	0.044	1.29	1.14-1.47
with clear HWE (n=8/8)	0.027	1.06	1.01-1.12	29.284	7	0.000	0.026	1.06	0.92-1.23
without clear HWE (n=10/7)	0.048	1.49	1.35-1.64	11.19	9	0.263	0.006	1.52	1.36-1.71
Case-control (n=20/14)	0.039	1.44	1.34-1.56	19.248	19	0.441	0.001	1.44	1.33-1.56
BY ADJUSTMENT FOR SMOKING									
Smoking adjusted (n=20/12)	0.042	1.44	1.32-1.56	20.241	19	0.380	0.003	1.43	1.31-1.57
Smoking not adjusted (n=19/18)	0.011	1.01	0.99-1.04	129.101	18	0.000	0.035	1.25	1.12-1.39
BY OCCUPATION									
Truck drivers (n=9/9)	0.051	1.47	1.33-1.62	8.369	8	0.398	0.001	1.47	1.33-1.63
Railroad Workers (n=6/6)	0.049	1.17	1.06-1.29	30.902	5	0.000	0.095	1.45	1.08-1.93
Mechanics (n=6/6)	0.061	1.27	1.13-1.43	14.968	5	0.010	0.059	1.35	1.03-1.78
Heavy equipment operators/dock workers(n=4/4)	0.034	1.1	1.03-1.18	8.016	3	0.046	0.033	1.28	0.99-1.66
Professional drivers and transportation operatives (n=6/6)	0.051	1.45	1.31-1.60	2.893	5	0.716	0	1.45	1.31-1.60
Professional drivers (n=4/4)	0.052	1.47	1.33-1.63	0.472	3	0.925	0	1.47	1.33-1.63
Transportation operatives (n=2/2)	0.2	1.19	0.81-1.76	1.4	1	0.237	0.086	1.3	0.70-2.43
Bus company workers* (n=4/4)	0.065	1.26	1.11-1.43	10.216	3	0.017	0.057	1.23	0.90-1.69
Bus drivers (n=2/2)	0.303	1.27	0.70-2.29	1.951	1	0.162	0.198	1.17	0.49-2.80
DE-grouped (n=5/5)	0.013	0.97	0.95-1.00	2.837	4	0.585	0	0.97	0.95-1.00

* Includes two (2) studies of bus mechanics (Rushton 1983, Gustavsson 1990) which are also included in the mechanics category.

Table C.3 Analysis of Studies of Diesel Exhaust Exposure and Lung Cancer: Results of Analysis Using Fixed- and Random-Effects Models (continued).

GROUP (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	τ^2	Random Effects Model Pooled RR	95% C.I.
BY LATENCY									
Latency clearly ≥10 yr. (17/16)	0.026	1.16	1.10-1.22	76.687	16	0.000	0.057	1.34	1.15-1.55
≥10 yr. exposure (9/8)	0.033	1.14	1.07-1.22	26.062	8	0.001	0.081	1.54	1.20-1.98
≥20 yr. exposure (6/6)	0.035	1.11	1.04-1.19	16.071	5	0.007	0.089	1.49	1.08-2.05
≤20 yr. exposure (3/2)	0.133	1.7	1.31-2.21	0.388	2	0.824	0	1.7	1.31-2.21
exposure duration not clear (8/8)	0.04	1.18	1.09-1.27	50.263	7	0.000	0.087	1.21	0.96-1.53
Latency not clearly ≥ 10 yr. (22/15)	0.012	1.01	0.99-1.04	116.551	21	0.000	0.056	1.34	1.17-1.54
BY REGION									
North America/USA (n=22/14)	0.028	1.18	1.12-1.25	68	21	0.000	0.062	1.45	1.25-1.69
Europe (n=17/16)	0.012	1.02	0.99-1.04	122.137	16	0.000	0.049	1.25	1.10-1.43
BY COMPARISON POPULATION									
Cohort studies									
Internal controls (n=6/4)	0.056	1.45	1.30-1.61	6.633	5	0.249	0.007	1.48	1.28-1.70
Regional/state (n=4/3)	0.08	1.21	1.04-1.42	26.878	3	0.000	0.238	1.4	0.83-2.39
National (n=8/8)	0.028	1.08	1.03-1.14	22.27	7	0.002	0.019	1.14	1.00-1.31

Table C.3 Analysis of Studies of Diesel Exhaust Exposure and Lung Cancer: Results of Analysis Using Fixed- and Random-Effects Models (continued).

GROUP (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
Case-control Studies									
Hospital controls (n=4/3)	0.01	1.25	1.03-1.51	2.124	3	0.547	0	1.25	1.03-1.51
Cancer controls (n=4/3)	0.139	1.6	1.22-2.11	8.209	3	0.042	0.161	1.85	1.11-3.09
General population (n=3/3)	0.182	1.05	0.73-1.50	1.315	2	0.518	0	1.05	0.73-1.50
Internal controls (n=5/4)	0.049	1.49	1.35-1.64	0.086	4	0.999	0	1.49	1.35-1.64
Multiple sources (n=4/1)	0.132	1.59	1.23-2.06	0.763	3	0.858	0	1.59	1.23-2.06
BY DURATION									
Exposure≥20 yr. (n=6/6)	0.035	1.11	1.04-1.19	16.071	5	0.007	0.089	1.49	1.09-2.05
Exposure < 20 yr. (n=11/6)	0.048	1.5	1.36-1.64	7.791	10	0.649	0	1.5	1.36-1.64
Exposure≥10 yr. (n=14/9)	0.032	1.17	1.10-1.25	37.38	13	0.000	0.073	1.61	1.31-1.98
Exposure < 10 yr. (n=3/2)	0.057	1.43	1.28-1.60	2.768	2	0.251	0.006	1.39	1.19-1.63
Exposure duration unclear (n=22/19)	0.012	1.01	0.98-1.03	122.228	21	0.000	0.048	1.24	1.10-1.40

Table C.4 Meta-analysis of Diesel Exhaust Exposure and Lung Cancer: Results of Subgroup Analysis Involving Stratification on Two Study Characteristics

GROUP (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
BY SMOKING AND OCCUPATION									
Truck drivers (n=9/9)	0.051	1.47	1.33-1.62	8.369	8	0.398	0.001	1.47	1.33-1.63
Adjusted for smoking (n=4/4)	0.091	1.48	1.24-1.77	4.867	3	0.182	0.023	1.53	1.20-1.94
Not adjusted for smoking (n=5/5)	0.061	1.46	1.30-1.64	3.483	4	0.480	0	1.46	1.30-1.64
Railroad Workers (n=6/6)	0.049	1.17	1.06-1.29	30.902	5	0.000	0.095	1.45	1.08-1.93
Adjusted for smoking (n=3/3)	0.138	1.68	1.28-2.19	1.437	2	0.487	0	1.68	1.28-2.19
Not adjusted for smoking (n=3/3)	0.053	1.11	1.00-1.23	21.517	2	0.000	0.097	1.27	0.88-1.86
Mechanics (n=6/6)	0.061	1.27	1.13-1.43	14.968	5	0.010	0.059	1.35	1.03-1.78
Adjusted for smoking (n=3/3)	0.163	1.22	0.88-1.67	2.238	2	0.327	0.015	1.25	0.87-1.80
Not adjusted for smoking (n=3/3)	0.066	1.28	1.13-1.46	12.639	2	0.002	0.091	1.41	0.94-2.12
Heavy equipment operators (n=4/4)	0.034	1.1	1.03-1.18	8.016	3	0.046	0.033	1.28	0.99-1.66
Adjusted for smoking (n=2/2)	0.356	2.43	1.21-4.88	0.0786	1	0.779	0	2.43	1.21-4.88
Not adjusted for smoking (n=2/2)	0.034	1.09	1.02-1.17	2.933	1	0.087	0.014	1.16	0.95-1.41
Professional drivers and transportation operatives (n=6/6)	0.051	1.45	1.31-1.60	2.893	5	0.716	0	1.45	1.31-1.60
Adjusted for smoking (n=4/4)	0.056	1.43	1.28-1.60	2.112	3	0.549	0	1.43	1.28-1.60
Not adjusted for smoking (n=2/2)	0.115	1.52	1.22-1.91	0.543	1	0.461	0	1.52	1.22-1.91
Bus company workers* (n=4/4)	0.065	1.26	1.11-1.43	10.216	3	0.017	0.057	1.23	0.90-1.69
Adjusted for smoking (n=1/1)	0.369	1.7	0.83-3.51	-	-	-	-	-	-
Not adjusted for smoking (n=3/3)	0.066	1.24	1.09-1.42	9.521	2	0.009	0.065	1.16	0.81-1.66
DE-Grouped (n=5/5)	0.013	0.97	0.95-1.00	2.837	4	0.585	0	0.97	0.95-1.00
Adjusted for smoking (n=3/3)	0.162	1.1	0.80-1.51	1.972	2	0.373	0	1.1	0.80-1.51
Not adjusted for smoking (n=2/2)	0.013	0.97	0.95-1.00	0.27	1	0.603	0	0.97	0.95-1.00
BY SMOKING AND DURATION									
Exposure ≥20 yr. (n=4/4)	0.13	1.64	1.28-2.11	3.202	3	0.362	0.0004	1.64	1.28-2.11
Exposure ≥10 yr. (n=11/6)	0.082	1.64	1.40-1.93	5.46	10	0.858	0	1.64	1.40-1.93
Exposure <10 yr. (n=3/2)	0.057	1.43	1.28-1.60	2.768	2	0.251	0.006	1.39	1.19-1.63
Duration not clear (n=7/5)	0.092	1.23	1.03-1.47	6.403	5	0.269	0.016	1.24	1.00-1.54

Table C.4 Meta-analysis of Diesel Exhaust Exposure and Lung Cancer: Results of Subgroup Analysis Involving Stratification on Two Study Characteristics (continued).

GROUP (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
BY DURATION and OCCUPATION									
Truck Drivers									
Exposure≥20 yr. (n=2/2)	0.23	2.41	1.53-3.81	0.009	1	0.924	0	2.41	1.53-3.81
Exposure < 20 yr. (n=2/2)	0.129	1.51	1.18-1.95	0.014	1	0.906	0	1.51	1.18-1.95
Duration not clear (n=5/5)	0.057	1.41	1.27-1.58	3.345	4	0.502	0	1.41	1.27-1.58
Railroad Workers									
Exposure ≥10 yr. (n=3/3)	0.117	1.76	1.40-2.21	1.458	2	0.482	0	1.76	1.40-2.21
Duration not clear (n=3/3)	0.054	1.07	0.96-1.19	14.588	2	0.000	0.072	1.19	0.84-1.68

* Includes two (2) studies of bus mechanics (Rushton 1983, Gustavsson 1990) which are also included in the mechanics category.

Table C.5 Sensitivity Analyses - Substitution of Excluded Redundant Studies

STUDIES (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
BY STUDY DESIGN									
Cohort[1] (n=17/14)	0.024	1.14	1.08-1.19	69.087	16	0.000	0.042	1.27	1.11-1.44
Case-control[2] (n=21/15)	0.039	1.45	1.35-1.57	20.335	20	0.437	0	1.45	1.35-1.57
Case-control[3] (n=21/15)	0.039	1.46	1.35-1.58	19.774	20	0.472	0	1.46	1.35-1.58
BY ADJUSTMENT FOR SMOKING									
Smoking adjusted[4] (n=21/12)	0.041	1.45	1.34-1.57	21.374	20	0.375	0.003	1.45	1.32-1.58
At least 10 yr. Exposure[5] (n=11/6)	0.078	1.63	1.40-1.90	7.92	10	0.637	0	1.63	1.40-1.90
>20 yr. Exposure[6] (n=4/4)	0.116	1.73	1.38-2.16	6.545	3	0.088	0.071	1.76	1.22-2.55
Unclear exposure duration[7] (n=8/6)	0.096	1.26	1.05-1.52	6.282	6	0.392	0.004	1.27	1.04-1.54
Not adjusted for smoking[8] (n=18/17)	0.011	1.01	0.99-1.03	112.771	17	0.000	0.032	1.22	1.09-1.36

1. Schenker 1984 substituted for Garshick 1988; minus Gustafsson 1986.
2. Damber and Larssen 1985 substituted for Damber and Larssen 1987; Emmelin added in place of Gustafsson in the cohort studies; Siemiatycki 1990 for Siemiatycki 1988; Burns and Swanson 1991 for Swanson 1993; Boffetta 1989 for Boffetta 1990
3. Damber and Larssen 1985 substituted for Damber and Larssen 1987; Emmelin added in place of Gustafsson in the cohort studies; Siemiatycki 1990 for Siemiatycki 1988; Burns and Swanson 1991 for Swanson 1993; but with Hall and Wynder 1984 for Boffetta 1990
4. Damber and Larssen 1985 substituted for Damber and Larssen 1987; Emmelin added in place of Gustafsson in the cohort studies; Siemiatycki 1990 for Siemiatycki 1988; Burns and Swanson 1991 for Swanson 1993; Boffetta 1989 for Boffetta 1990
5. Burns and Swanson 1991 for Swanson 1993; Damber and Larssen 1985 for Damber and Larssen 1987; Boffetta 1989 for Boffetta 1990.
6. Burns and Swanson 1991 for Swanson 1993; Damber and Larssen 1985 for Damber and Larssen 1987; Boffetta 1989 for Boffetta 1990.
7. Added Emmelin 1993; Siemiatycki 1990 for Siemiatycki 1988.
8. Schenker 1984 for Garshick 1988; minus Gustafsson 1986.

Table C.6 Influence Analyses - Pooled RR Estimates for Lung Cancer in Selected Diesel-Exposed Occupations Deleting Single Studies

STUDIES (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
BY OCCUPATION									
Truck Drivers									
All Studies	0.051	1.47	1.33-1.62	8.369	8	0.398	0.001	1.47	1.33-1.63
Minus Ahlberg	0.064	1.56	1.37-1.77	6.096	7	0.529	0	1.56	1.37-1.77
Minus Balarajan	0.052	1.46	1.32-1.62	8.242	7	0.312	0.004	1.48	1.32-1.67
Minus Boffetta	0.054	1.5	1.35-1.67	6.923	7	0.437	0	1.5	1.35-1.67
Minus Hansen	0.056	1.44	1.29-1.60	7.691	7	0.361	0.003	1.46	1.29-1.64
Minus Hayes	0.054	1.46	1.32-1.62	8.344	7	0.303	0.005	1.49	1.31-1.69
Minus Menck	0.052	1.45	1.31-1.61	7.957	7	0.336	0.003	1.47	1.31-1.65
Minus Rafsson	0.051	1.46	1.32-1.61	7.33	7	0.395	0.001	1.46	1.32-1.62
Minus Steenland	0.052	1.46	1.32-1.62	8.311	7	0.306	0.005	1.48	1.32-1.67
Minus Swanson	0.052	1.44	1.30-1.59	4.716	7	0.695	0	1.44	1.30-1.59
Railroad Workers									
All Studies	0.049	1.17	1.06-1.29	30.902	5	0.000	0.095	1.45	1.08-1.93
Minus Boffetta	0.05	1.16	1.05-1.27	29.527	4	0.000	0.101	1.43	1.04-1.96
Minus Swanson	0.05	1.15	1.04-1.27	26.239	4	0.000	0.085	1.36	1.02-1.82
Minus Garshick 87	0.051	1.14	1.03-1.26	28.233	4	0.000	0.105	1.43	1.03-1.99
Minus Garshick 88	0.051	1.12	1.02-1.24	23.65	4	0.000	0.086	1.37	1.01-1.86
Minus Howe	0.059	1.1	0.98-1.23	27.211	4	0.000	0.166	1.51	1.01-2.26
Minus Nokso-Koivisto	0.069	1.5	1.31-1.71	4.729	4	0.316	0.006	1.53	1.31-1.80
Mechanics									
All Studies	0.061	1.27	1.13-1.43	14.968	5	0.010	0.059	1.35	1.03-1.78
Minus Benhamou	0.065	1.3	1.14-1.47	13.94	4	0.007	0.075	1.47	1.05-2.05
Minus Hayes	0.062	1.26	1.12-1.42	13.685	4	0.008	0.06	1.31	0.98-1.74
Minus Menck	0.062	1.25	1.11-1.41	10.538	4	0.032	0.041	1.25	0.98-1.61
Minus Steenland	0.062	1.27	1.12-1.43	14.83	4	0.005	0.067	1.35	1.00-1.82
Minus Gustavsson	0.084	1.1	0.94-1.30	9.09	4	0.059	0.077	1.33	0.94-1.90
Minus Rushton	0.077	1.45	1.25-1.69	6.74	4	0.150	0.04	1.49	1.12-1.99

Table C.6 Influence Analyses - Pooled RR Estimates for Lung Cancer in Selected Diesel-Exposed Occupations Deleting Single Studies (continued)

STUDIES (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
BY OCCUPATION									
Professional Drivers and Transportation Operatives									
All studies	0.051	1.45	1.31-1.60	2.893	5	0.716	0	1.45	1.31-1.60
Minus Damber	0.051	1.46	1.32-1.61	2.56	4	0.634	0	1.46	1.32-1.61
Minus Guberan	0.056	1.44	1.29-1.60	2.787	4	0.594	0	1.44	1.29-1.60
Minus Pfluger	0.08	1.4	1.20-1.64	2.621	4	0.623	0	1.4	1.20-1.64
Minus Berhamou	0.054	1.45	1.31-1.61	2.872	4	0.579	0	1.45	1.31-1.61
Minus Buiatti	0.052	1.47	1.33-1.63	1.082	4	0.897	0	1.47	1.33-1.63
Minus Wegman	0.051	1.44	1.31-1.59	2.24	4	0.692	0	1.44	1.31-1.59

Table C.7 Sensitivity Analysis - Pooled RR Estimates Excluding Studies with Uncertain Exposures to Diesel Exhaust*

STUDIES (# risk estimates/# studies)	Fixed Effects Model se	Pooled RR	95% C.I.	Q-statistic	df	p-value	Random Effects Model τ^2	Pooled RR	95% C.I.
ALL STUDIES (n=29/24)	0.011	1.03	1.01-1.06	184.835	28	0.000	0.042	1.35	1.22-1.49
Cohort studies (n=16/13)	0.024	1.17	1.11-1.22	62.122	15	0.000	0.037	1.33	1.18-1.51
With clear HWE (n=6/6)	0.028	1.07	1.02-1.13	16.687	5	0.005	0.016	1.1	0.96-1.25
Without clear HWE (n=10/7)	0.048	1.49	1.35-1.64	11.19	9	0.263	0.006	1.52	1.36-1.71
Case-control studies (n=12/10)	0.045	1.47	1.34-1.60	12.823	11	0.305	0.005	1.46	1.31-1.63
Smoking-adjusted (n=13/9)	0.049	1.47	1.33-1.61	14.519	12	0.269	0.009	1.47	1.29-1.67
Smoking-adjusted									
Duration $\geq$20 yr. (n=4/4)	0.128	1.64	1.28-2.11	3.202	3	0.362	0.005	1.64	1.26-2.14
Duration $\geq$10 yr. (n=7/5)	0.104	1.68	1.37-2.06	4.606	6	0.595	0	1.68	1.37-2.06
Duration < 10yr (n=1/1)	0.065	1.48	1.30-1.68	--	--	--	--	--	--
Duration unclear (n=5/3)	0.102	1.26	1.03-1.54	6.064	4	0.194	0.032	1.28	0.97-1.69
Not adjusted for smoking (n=16/15)	0.011	1.01	0.99-1.04	115.961	15	0.000	0.033	1.27	1.14-1.43
Truck drivers (n=7/7)	0.055	1.45	1.31-1.62	8.207	6	0.223	0.009	1.5	1.30-1.73
Mechanics (n=4/4)	0.065	1.29	1.13-1.46	12.76	3	0.005	0.078	1.41	0.99-2.02
Prof'l Drivers/ Transportation (n=3/3)	0.056	1.48	1.32-1.65	0.412	2	0.814	0	1.48	1.32-1.65

* Benhamou (2 estimates), Buiatti, Kauppinen, Wegman and Peters, Hayes (4 estimates), Bender, and Balarajan

Figure C-1: Estimates of Relative Risks for Occupational Categories: Truck Drivers

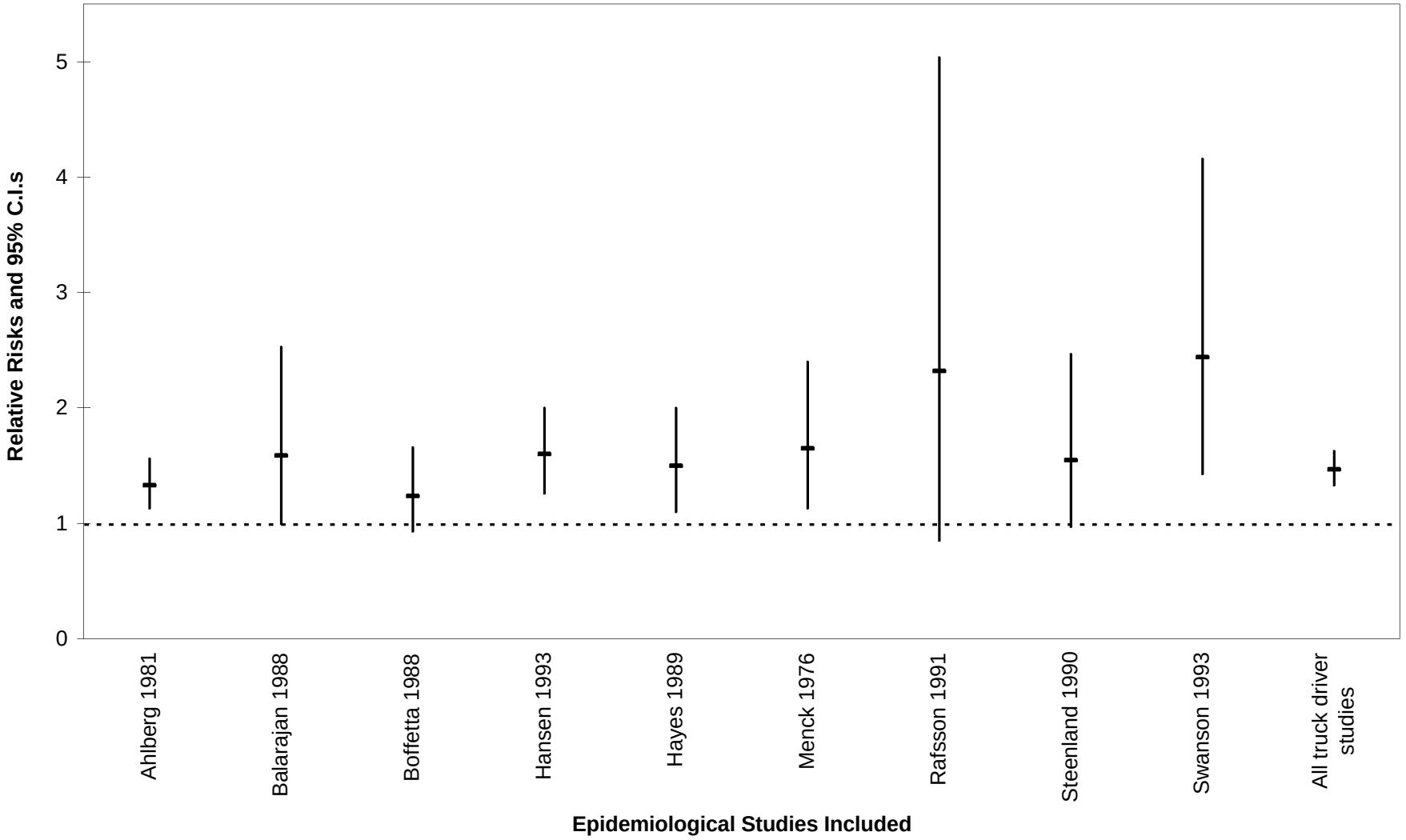


Figure C-2: Estimates of Relative Risks for Occupational Categories: Railroad Workers

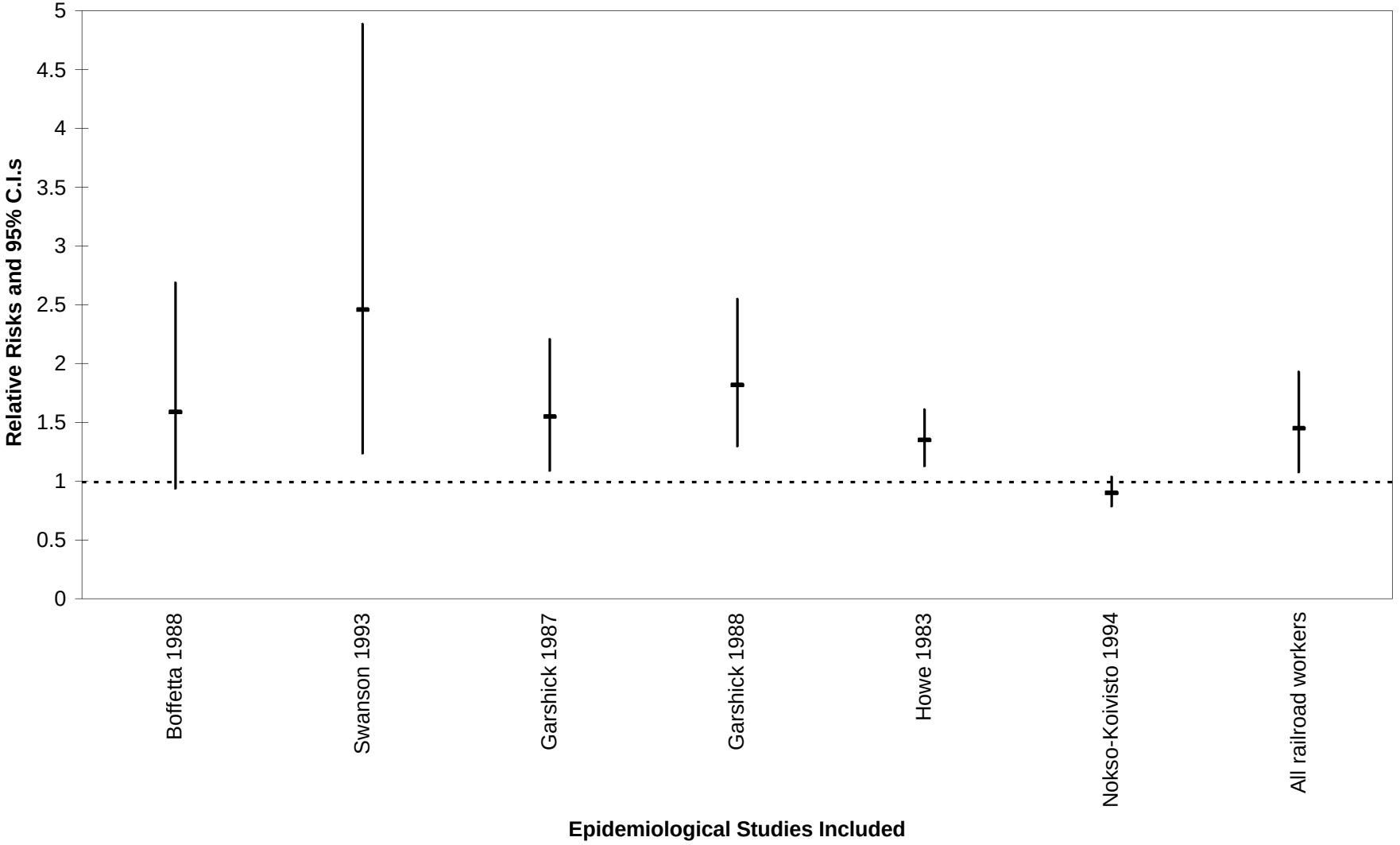


Figure C-3: Estimates of Relative Risks for Smoking-Adjusted Studies of Diesel Exhaust Exposure and Lung Cancer

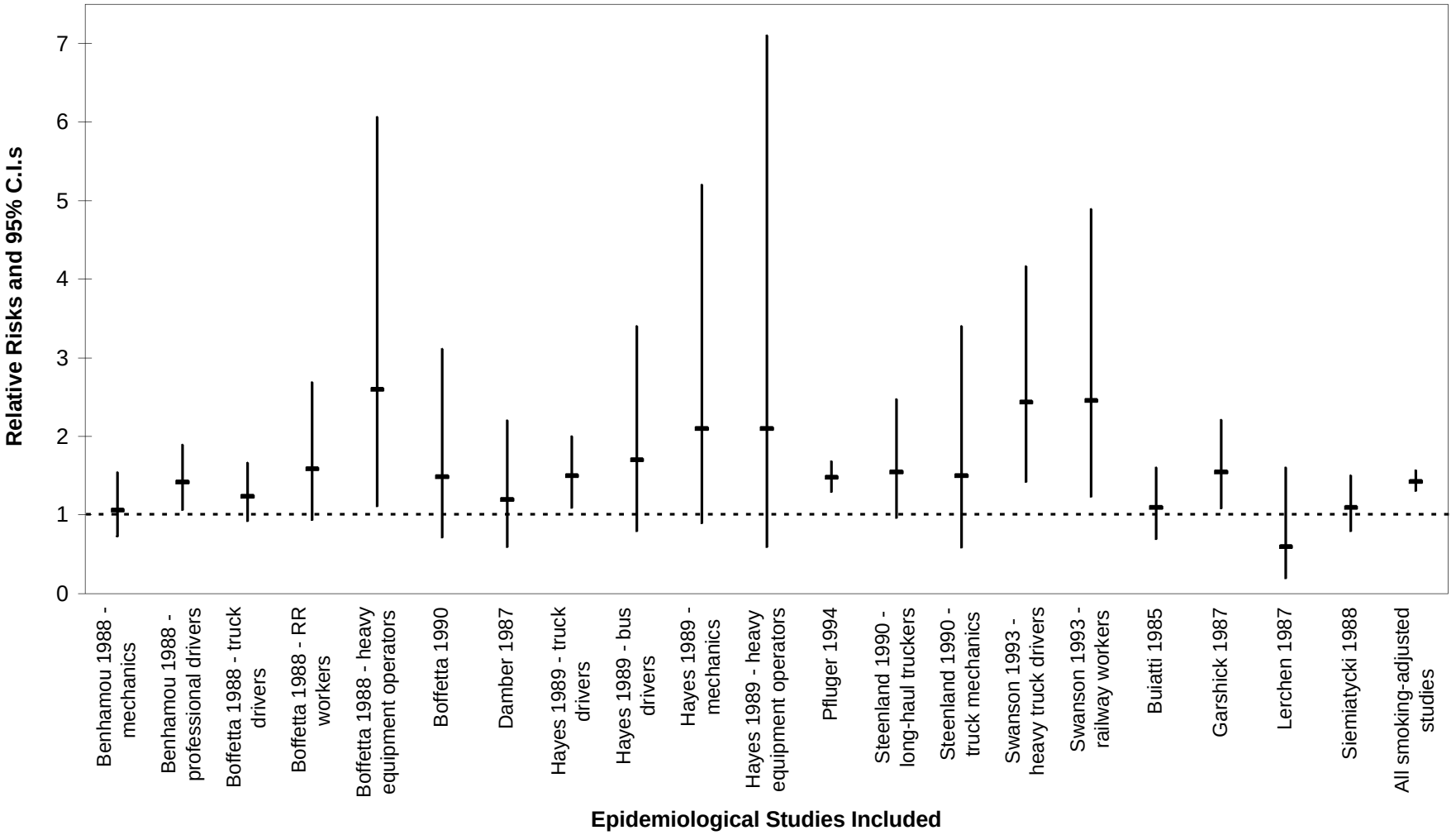


Figure C-4: Estimates of Relative Risks for Studies of Diesel Exhaust Exposure and Lung Cancer that were not Adjusted for Smoking

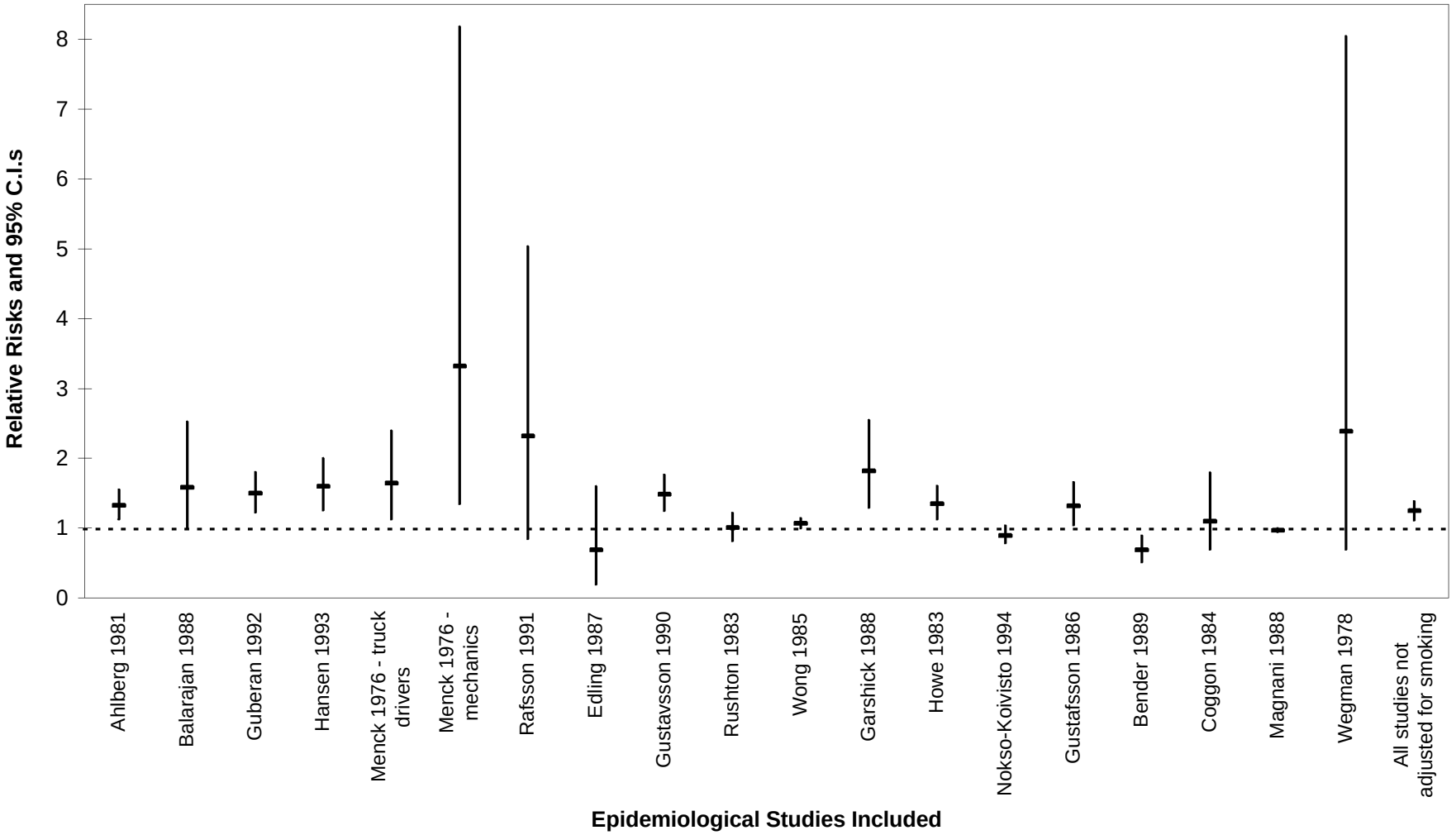
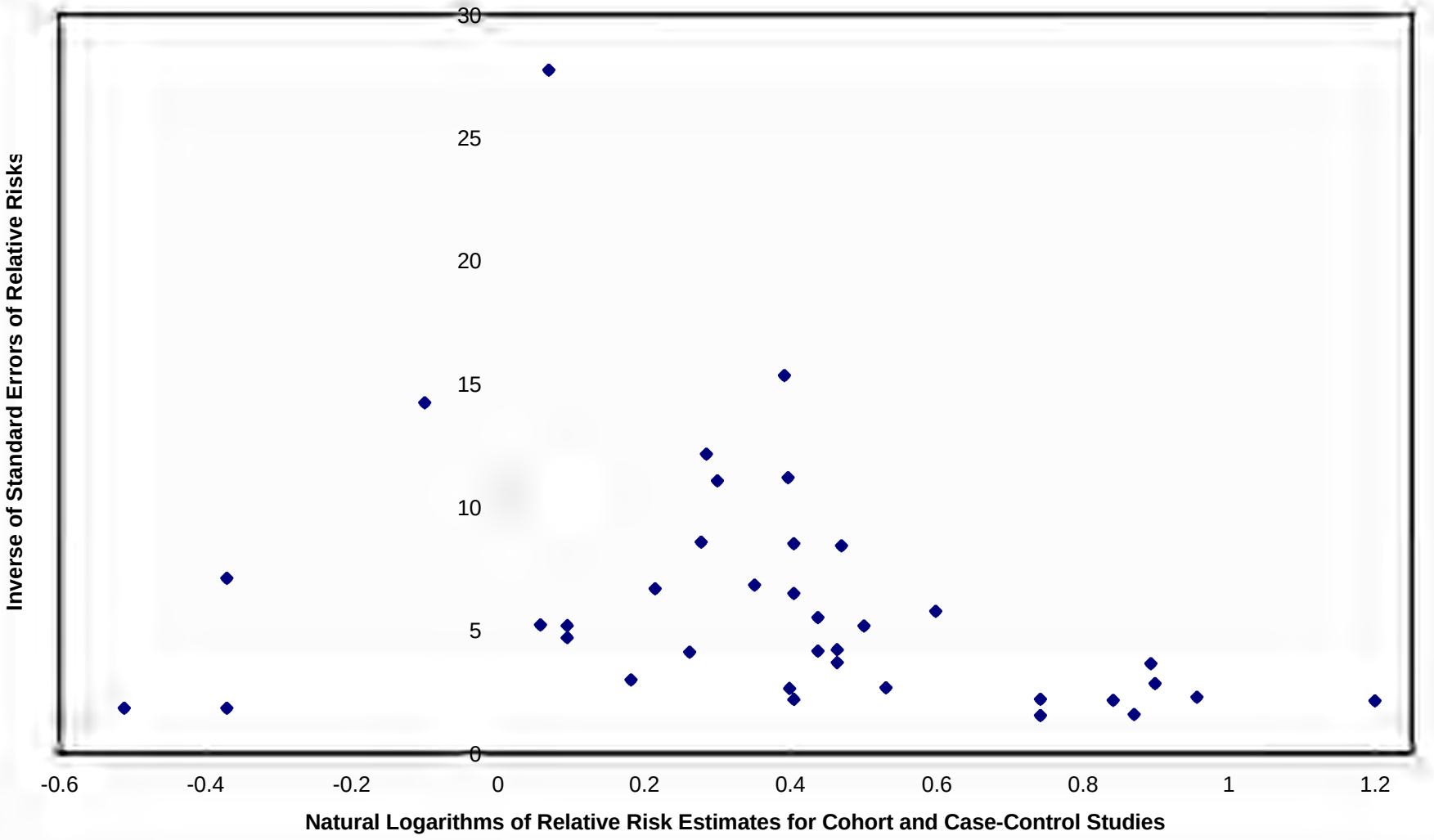


Figure C-5: Plot of Natural Logarithms of Relative Risk Estimates Versus the Inverse of Their Standard Errors



APPENDIX D

CALCULATIONS OF RELATIONSHIP OF RISK TO DIESEL EXHAUST EXPOSURE, USING THE INDIVIDUAL DATA USED IN GARSHICK *ET AL*. (1988)

The Health Risk Assessment for Diesel Exhaust (Office of Environmental Health Hazard Assessment, Cal/EPA, 1994 draft) provided an assessment of risk of diesel exhaust based on published values of relative hazard in the railroad worker cohort study of Garshick *et al*. (1988). The assessment did not refer to the reanalysis in an unpublished report of Crump *et al*. (1991) because that report contained two sets of analyses that appeared to contradict each other and because it had not received the scrutiny of publication. Cal/EPA subsequently received public comments pointing to the significantly negative slopes of risk against exposure found in that report and the conclusion of the authors that "No relationship between measures of diesel exposure and lung cancer mortality is demonstrated in this study."

The OEHHA staff pursued an investigation, using the original individual data, to resolve the apparent contradiction in the Crump *et al*. report. After correcting a misclassification of deaths in exposure categories used for the report, the investigation determined in parallel calculations with Dr. Crump, that the particular approach of the report still did not lead to a significantly positive relationship. The investigation then explored reasonable alternative approaches that might lead to a significantly positive relationship. The main results of that investigation are presented below. During this investigation Dr. Crump has been most cooperative in furnishing information and calculations and in responding to preliminary materials. See Appendix E for a summary of unresolved issues that have emerged during this time and for a list of the correspondence cited in this appendix.

In order to obtain unit risk slopes for quantitative risk assessment, this appendix presents staff calculations of the relationship of risk to diesel exhaust exposure, using the individual data used in the Garshick *et al*. (1988) cohort study of US railroad workers. The analyses use two different assumptions about the time pattern of exposure and two different types of models in order to explore how uncertainties in these choices influence the calculated risks.

The sections below present the methods and the results and a discussion of the results.

D.1 DESCRIPTION OF THE STUDY

To develop the original data set, Garshick *et al*. (1988) obtained the following information for each individual in their cohort of railroad workers for the follow-up years of 1959-1980: cause of death by death certificate, the primary job classification for each year, and months worked in that classification in each year. In addition they obtained the age at the start of follow-up in 1959, total service months and, only for those workers who began work after 1946, the date of starting work. Woskie *et al*. (1988a,b) reported measurements of diesel exhaust in a number of different railroad work places shortly after the end of follow up in the study.

D.2 THE ANALYSES FOR FOUR DIFFERENT COMBINATIONS OF ASSUMPTIONS

The analyses for the four (2 x 2) different combinations of assumptions use: (1) two different assumptions about the exposure pattern -- one with a ramp-shaped time dependence, the other with a (peaked) roof shape of time dependence -- and (2) two different model types to control for the effect of age and time -- a set of general log-linear epidemiological models and the multistage model of Armitage and Doll, which is specialized for cancer.

D.2.1 METHODS FOR THE ANALYSES

Exposure

Woskie *et al*. (1988a) obtained measurements of concentration of respirable particulate matter (RSP), adjusted for environmental tobacco smoke (ETS), for the relevant jobs in a sample of U.S. railroads. Woskie *et al*. (1988b) expressed these measurements as national career group means. Exposure to diesel exhaust follows by subtracting the particle measurements of the workers not exposed to diesel exhaust, clerks and signal maintainers, from the particle measurements of each exposed group. The round numbers used here for the ETS-adjusted RSP concentrations are 50 $\mu g/m^3$ for the clerks and signal maintainers and 100 $\mu g/m^3$ for the train workers and 150 $\mu g/m^3$ for exposed shop workers. The analyses below exclude shop workers because of the apparent heterogeneity of their exposure. A substantial but unknown number did not work in diesel-engine repair shops which experienced the high concentrations.

Woskie *et al*. (1988b) obtained these measurements in the early 1980's, just after the follow-up of the railroad worker cohort ended, and made general remarks about historical exposures. Some specific assumptions are required to reconstruct approximate exposure histories for the railroad workers. As part of this approximation, all the analyses assume that exposure concentration rose linearly from zero in 1945 to the early 1980's value in 1959. This increasing portion of the exposure pattern is consistent with the data cited in Woskie *et al*. (1988b) about the rate of conversion of the locomotive fleet to diesel in the United States. Subsequent to 1959, two different assumptions are made in the calculations below about the time course of the exposure, which is much less certain. One assumption, suggested by Woskie *et al*. (1988b) in the absence of contradictory data, is that the concentration remained constant through the follow-up period, 1959-1980. This assumption together with the assumption of linear increase in the prior period leads to the "ramp" pattern of exposure, introduced by Crump *et al*. (1991). The other assumption is that after the initial linear increase, exposure peaked in 1959 at a value that is three times the value in 1980 and subsequently declined linearly to the 1980 value. This (peaked) "roof" approximation is consistent with anecdotal accounts of an early era of smokier engines (Woskie *et al*. 1988b), the end of which "may have decreased diesel exposures of train crews over time." Co-author K. Hammond (oral communication) reports that the smokier (ALCO) engines were shifted from the main lines to the yards over this time, resulting in less exposure to the bulk of the train workers. The ramp approximation can be regarded as a lower bound on the time-dependent pattern of concentration, ultimately giving an upper bound on risk. The roof

approximation is likely to be nearer the actual pattern. Figure D-1 shows both the roof pattern and the ramp pattern.

Calculations

In all the analyses the process of fitting the model to the data determines the parameters of the model. The general model uses eleven different covariate structures to attempt to control for the well known effects of age and calendar year. All assume that the effect of exposure on incidence is measured by the area under the curve of past concentration up to the lag time from carcinogenesis to death, as in Crump *et al*. (1991). The multistage model, which assumes that the number of stages is to be determined, has a specified form of relationship for age and for weighting concentration as a function of age in order to determine incidence.

The initial step in the data reduction by DATAB in EPICURE (Preston *et al*. 1993) is to specify category boundaries for the independent variables in the analysis and then to count numbers of cancer deaths, and numbers of person years at risk and to calculate mean values of the four continuous variables that are given for each individual, three during each year of follow-up -- measure of exposure, age attained at each year of follow-up, calendar year -- and one from age at start of the study -- birth year. For these age and temporal variables the choices of category boundaries are made as follows: attained age, 10 year categories; age at start of study, 5 year categories; calendar year, 4 year categories except for the last category, which has only 2 remaining years; exposure, (a) the number of categories chosen to give a smooth trend with the best chance of statistical significance of the slope to be calculated and (b) category boundaries giving approximately equal numbers of cancer deaths in each category. The use of 10-year categories for attained age is more computationally convenient than the usual use of 5-year categories, and both these categorizations gave essentially the same relationships of risk with exposure in pilot calculations.

All these analyses exclude the last 4 years of follow up because of evidence of under-reporting of deaths in those years, first pointed out in Crump *et al*. (1991). The analyses that provide all the primary results exclude shop workers because a substantial though unknown number of shop workers apparently had no diesel exposure.

After the initial step of cross tabulation by category of exposure, attained age, start age (age at start of follow up, in 1959, reflecting birth cohort) and calendar year, the selected models are fit to the cross-tabulated data by Poisson regression (Breslow and Day, 1987, pp. 120-142), using AMFIT in EPICURE. This program determines the open parameters in each model by maximizing the likelihood function (minimizing the deviance between observed and calculated incidences) for the Poisson probability of the model prediction of incidence of lung cancer, given the cross-tabulated form of the data.

Where practical, formal criteria for goodness of fit are used to indicate preferred models.

Exploring trends of incidence with exposure

As a primary way to characterize the trend of incidence with exposure, all the analyses use a log-linear assumption, which is essentially linear over the small range of low exposures of these workers. As a secondary way to characterize the trend of incidence with exposure, all the analyses use a segmented scale defined by the exposure categories, producing a stepped form of trend of incidence with exposure.

The estimates of exposure slope for the log-linear trend provide the basis for the cancer potency sought for the quantitative risk assessment. For each model which is log-linear in exposure, the calculations determine the maximum likelihood estimate (MLE) of the exposure slope, its standard error, and its upper and lower 95% confidence limit.

Results for the categorical trend with exposure allow a visual check and a quantitative check on significant departures of the trend from log-linearity. Because the slope model is essentially nested in the categorical model, the difference between the deviance for each model with categorical characterization of exposure and the deviance of the corresponding slope model is approximately distributed like chi-squared. The degrees of freedom are equal to the difference of degrees of freedom between the corresponding categorical and the slope models. On this basis, p-values of significant differences of fit are readily calculated.

Unit risks

Unit risk is estimated as the risk of lung cancer from breathing air contaminated at the concentration of $\mu g/m^3$ (one unit of concentration) for a lifetime. To obtain unit risks from the slopes determined for hazard with exposure in these analyses the following procedures are used:

(1) The correction due to intermittency of exposure during the calendar year is applied by multiplying the exposure:

$$(48 \text{ wk} / 52 \text{ wk}) * (5 \text{ d} / 7 \text{ d}) * (10 (m^3/\text{d}) / 20 (m^3/\text{d})) = 0.33$$

Thus, as used below, the term working year is for 0.33 of a calendar year of exposure.

(2) The theoretical absolute risk (probability) of lung cancer due to exposure is obtained by using a life table calculation. This calculation starts with a background life table for lung cancer in California (Table D-1). For each unit risk to be calculated, a modification of that table is constructed in a way that includes the predicted effect of a lifetime exposure to 1 unit of concentration, 1 $\mu g/m^3$ in the present calculations. The predicted effect is incorporated by multiplying the background lung cancer incidence for each age interval in the table by the risk (relative hazard) for that age interval. See, for example, Table 7-8. The relative risk is (1+ excess relative risk due to exposure). The excess relative risk due to exposure for unit concentration is the slope of relative risk with concentration, obtained from the epidemiological analyses. Using the general model based on cumulative exposure, as in the present calculations,

the excess relative risk requires the slope coefficient per concentration-yr to be multiplied by the age in years for each age group in the table and to be divided by the intermittency factor. The multistage model slope coefficients are simply per concentration and do not require multiplication by age, simply division by the intermittency factor. Any ages that fall within the number of years of detection lag prior to the target age have zero excess relative hazard. The modified table is completed in the manner of the original table. The lifetime unit risk is then the following difference: the probability of lung cancer at the target age in the table modified by exposure less the probability at the same age in the original table.

The MLE value and the 95% UCL value of unit risk are presented in Table D-4 for each of the combinations of different assumptions used in the calculations.

D.2.2 THE ANALYSES USING THE GENERAL MODELS

For both the analyses, the standard categorizations are as follows:

attained age -- four or five 10-yr intervals,
start age (birth cohort) -- five 5-yr intervals,
calendar year -- five 4-yr intervals,
cumulative exposure -- four to six categories with each exposed category having equal cancer deaths.

The same categorizations were used in the model fitting as for the initial cross-tabulation.

To explore different ways of accounting for effects of age and calendar year the analyses of both combinations use the following models, eleven in all, that are continuous in cumulative exposure. Each analysis also uses the corresponding models that characterize cumulative exposure by category. Two of these general models use external standardization, five use different internal categorical covariate combinations and four use different internal continuous covariate combinations.

Categorical covariates, external:

#1 Prate(k,i) = K0* Nrate(k,i)* exp(S*cumx),
#2 Prate(k,i,j) = K0* Nrate(k,i)* exp(Byr(j)+ S*cumx).

Categorical covariates, internal:

#3 Prate(k,i) = Prate0* exp(Cyr(k)+ Aage(i)+ S*cumx),
#4 Prate(k,j) = Prate0* exp(Cyr(k)+ Byr(j)+ S*cumx),
#5 Prate(k,i,j) = Prate0* exp(Cyr(k)+ Aage(i)+ Byr(j)+ S*cumx),
#6 Prate(k,j) = Prate0* exp(CyrByr(k,j)+ S*cumx),
#7 Prate(k,i) = Prate0* exp(CyrAage(k,i)+ S*cumx).

Continuous covariates, internal:

#8 Prate(aage) = Prate0* exp(A1*aage+ A2*aage^2+ S*cumx),
#9 Prate(cyr,aage) = Prate0*
exp(C1*cyr+ C2*cyr^2+ A1*aage+ A2*aage^2+ S*cumx),
#10 Prate(cyr,byr) = Prate0*
exp(C1*cyr+ C2*cyr^2+ B1*byr+ B2*byr^2+ S*cumx),

#11 Prate(cyr,byr) = Prate0*
*(C1*cyr + C2*cyr^2 + B1*byr + B2*byr^2 + CB*cyr*byr + S*cumx),

where

Prate(argument) = the lung cancer rate for the covariate argument indicated,
K0 = a proportionality constant,
Nrate = the US rate,
cumx = the cumulative exposure, a continuous variable,
Q_1 = the slope for the cumulative exposure,
Prate0 = the baseline lung cancer rate, to which relative hazard refers,
Aage(i) = Ln of the relative hazard for attained-age category i,
Byr(j) = Ln of the relative hazard for start age (birth cohort) category j,
Cyr(k) = Ln of the relative hazard for calendar year category k,
CyrAage(k,i), CyrByr(k,j) = Ln's of the relative hazard for the indicated interactions,
aage, byr, cyr are the indicated continuous variables,
A1, A2, B1, B2, C1, C2, CB are parameters to be determined for the continuous variables (lower case)
Ln = natural logarithm.

Using the usual convention, the relative hazards are taken with respect to category 1 for each categorical variable; so the relative hazard for category 1 is 1.

These models provide a considerable range of assumptions about the covariate time-dependent variation in the modeling process. The simpler of the externally standardized models makes rigid assumptions about the form of the variation and has fewest parameters to determine. The models using continuous covariates in quadratic forms are rather constrained and are conceptually appealing in requiring that the time variation of the rates be smooth. The models with independent multiplicative categorical variables provide increased flexibility of the form to fit and thus have a larger number of parameters to determine. The interactive model allows independent variation of each of the categorical elements in its matrix, which cause it to produce the most parameters to estimate.

All the results presented for these general models assume a 5-year lag from carcinogenesis to death. This is the lag found by Garshick *et al*. (1988) to give a significant trend of relative hazard with cumulative exposure, using time in the years of follow up. Some pilot explorations confirm that this lag was most useful in the present analyses.

Goodness of fit

For each model in an analysis, the deviances and degrees of freedom are used to calculate two different criteria, AIC and BIC, for selecting the best fitting model by choosing the maximum value of each (Wang *et al*. 1996). The results below use the following modified formulas that differ by an inessential constant from the original definitions, for convenience in this analysis:

AIC = df - n - 0.5*md,

BIC = (df - n)*0.5*ln(n) - 0.5*md,

where

n = total number of data points (cells) in the analysis,
df = number of degrees of freedom,
md = deviance corresponding to the MLE, defined as

- 2*[ln(MLF) - ln(SLF)],

where

MLF = maximum likelihood function,
SLF = likelihood function evaluated for saturation, for which the prediction of incidence in each cell equals the observed rate.

Both criteria are weighted towards utilizing fewer parameters in the model. BIC places more weight on having fewer parameters than AIC (Wang *et al*. 1996).

D.2.2.1 RESULTS FOR GENERAL MODELS WITH ROOF EXPOSURE PATTERN

The exposure-dependent results of analyses for the general model with the roof exposure pattern are summarized in Table D-2. The MLE values for the slopes for the incidence as a function of cumulative exposure range from 8.6×10^{-5} to 2.3×10^{-4} (relative hazard per work yr-µg/m^3)$^{-1}$. The slope coefficients are all highly significant statistically. The values of AIC and BIC in the table suggest a way to choose a preferable model based on these measures of penalized fit. The three highest values of AIC, which are substantially above the rest, are for (1) the model using external rates and birth year (Nrate+Byr), (2) the model using all three categorical age-time controls (Byr+Cyr+Aage) and (3) the model for continuous values of birth cohort and calendar year (byr+byr^2+cyr+cyr^2+byr*cyr). The two highest values of BIC, which are definitely above the others, are for the two models using external rates (Nrate and Nrate+Byr). The overlap of high values in the AIC and BIC results suggests that the model using external rates and birth year is preferable from the point of view of fit of the model to the data. This preference is supported by the chi-squared (deviance) statistic showing a highly significant improvement in fit for that model in comparison to the model using external rates alone. This preference is also helpful in resolving any question about problems of colinearity of time variables that can be troubling if they have to be used simultaneously as explanatory variables. Birth year appears to be important in taking account of how this cohort differed from the national cancer statistics.

The preferable model, using national rate and birth cohort (Nrate+Byr), has an MLE slope, Q_1, of 1.2×10^{-4} (95% CI: 6.0×10^{-5} to 1.7×10^{-4}) for relative hazard per working yr-µg/m^3. Correcting for intermittency during the working year and converting from the annual rate of relative risk to a lifetime absolute risk using a life table yields a 95% UCL of the unit risk of $q_1^* = 7.2 \times 10^{-4}$ (lifetime-µg/m^3)$^{-1}$. By a similar calculation the MLE value is $q_1 = 5.1 \times 10^{-4}$ (lifetime-µg/m^3)$^{-1}$.

Also shown in the table (last column) are the p-values for the improvement in fit using categorical exposure instead of the slope of the continuous log-linear relationship. In all cases,

using the categorical form improves the fit significantly. Figure D-2 shows the relationship between the log-linear model selected and the corresponding categorical form. Although there appears to be a definite positive relationship between risk and diesel exhaust, the departure from log-linearity is marked; so the log-linear description of that relationship as determined by this model must be considered very approximate.

D.2.2.2 RESULTS FOR GENERAL MODELS WITH RAMP EXPOSURE PATTERN

The exposure-dependent results of analyses for the general model with the ramp exposure pattern are summarized in Table D-3. The MLE values for the slopes for the incidence as a function of cumulative exposure range from 2.1×10^{-4} to 3.7×10^{-4} (relative hazard per work yr-μg/m^3)$^{-1}$. The slope coefficients are all significant statistically, though not nearly as much so as for the roof pattern (Table D-2). The values of AIC and BIC in the table have virtually the same relationship as those for the roof pattern. Using these criteria of penalized fit as with the roof pattern, the preferred slope is that calculated for the model with national rate and birth cohort (Nrate+Byr), which is selected as the simplest model to explain the data. It has an MLE slope , Q_1, of 2.9×10^{-4} (95% CI: 1.3×10^{-4} to 4.4×10^{-4}) relative hazard per work yr-μg/m^3. Thus, the 95% UCL of the unit risk for this model is $q_1^* = 1.9 \times 10^{-3}$ (lifetime-μg/m^3)$^{-1}$, and the MLE value is $q_1 = 1.2 \times 10^{-3}$ (lifetime-μg/m^3)$^{-1}$, corrected for intermittency during the working year and converted to absolute risk, as above.

Table D-3 shows that a categorical characterization of trend of incidence provides a statistically significant improvement of fit over the log-linear relationship in the case of four out of eleven models, including the preferred model (Nrate+Byr). Figure D-3 shows a comparison between the log-linear and categorical forms of this model. Because of the potential departure from log-linearity, the log-linear relationship must be regarded as possibly being quite approximate.

D.2.3 ANALYSES USING THE MULTISTAGE MODEL

These analyses use a multistage model of carcinogenesis, which takes into account the age at which exposures occur. The use of the Armitage-Doll form of the multistage model was suggested in a review (Thomas, 1994, correspondence) of an earlier draft of the quantitative risk assessment. It is based on accepted mechanisms of carcinogenesis and has its own specific age dependence. Thus, among other advantages, it is not subject to the questions about correlated time variables that occur for the general models. Armitage and Doll (1961) proposed the model, by which k cellular transformations give rise to k cell stages, and the final stage is a cancerous cell. Whittemore (1977) and Thomas (1982) evolved the mathematical form used in these analyses, in which a single transformation, number i in the sequence of transformations, is attributed to diesel exhaust and the rest are attributed to background exposures. Brown and Chu (1982) and Crump and Howe (1984) developed related analyses. The resulting hazard or prediction of tumor appearance rate (incidence), h(t), at age t is the following:

$$h(t) = \begin{cases} \{ a_1 \ldots a_k (t-z)^{k-1} / (k-1)! \} \{ 1 + (b_i/a_i)\, I(t-z;k,i) \}, & i = 1,..,k-1 \\ \{ a_1 \ldots a_k (t-z)^{k-1} / (k-1)! \} \{ 1 + (b_i/a_i)\, c(t-z) \}, & i = k, \end{cases}$$

where

$$I(s;k,i) = I_0^{\,s} (s-u)^{k-i-1} u^{i-1} c(u)\, du\, (k-1)! / [\, s^{k-1} (k-i-1)!\, (i-1)!\,]$$

is a normalized Armitage-Doll exposure integral with the single transformation, order number i, being dependent on the concentration of the contaminant under investigation, in this case diesel exhaust,

a_j = coefficients for concentration in the transformation rates of the preceding stage due to background, j=1,..,k,

b_i = coefficient for the transformation rate of the preceding stage that depends on the concentration of the contaminant, and

z = time lag from carcinogenesis to death.

In fitting the data, the analysis determines the two transformation numbers, i and k, and the two unknown overall coefficients, which are the combined quantities,

$a_1 \ldots a_k / (k-1)! = Q_0$, the coefficient of t^{k-1} for background incidence, independent of diesel exhaust concentration, and

$b_i/a_i = R_1$, for the relative rate of the transformation that depends on diesel exhaust concentration $(\mu g/m^3)^{-1}$.

Thus, for all forms of the model the overall coefficient (slope) for the relative concentration-dependent effect, when divided by the proportion accounting for intermittency of the work year, is R_1.

Implementation of the model also considered the multiplicative use of covariates, calendar year (Cyr) and age-at-start-of-study (Byr).

The lifetime risk of lung cancer is then obtained from the integral (I) of the hazard:

$$I_0^{\,T} h(t)\, dt = Q_0 \{ I_z^{\,T} (t-z)^{k-1} dt + R_1 I_z^{\,T} (t-z)^{k-1} I(t-z;k,i) dt \},$$

for values of this intergral that are small compared to one, as in the present work.

For $c(t) = c_0$ = constant over lifetime, $I(s;k,i) = c_0$ (Dwight, 1961: #853.21, obtained by taking c_0 outside the integral that defines I and changing the dummy variable to x = u/s). Then the lifetime risk becomes

$$\{ Q_0 (T-z)^k / k \} \{ 1 + R_1 c_0 \}.$$

The quantity on the right is the relative risk (relative hazard). With $c_0 = 1\ \mu g/m^3$ this is the quantity (R_1) to multiply the incidences in the life table by to obtain the lifetime unit risk of lung cancer in the target population.

D.2.3.1 RESULTS FOR THE MULTISTAGE MODEL

Fitting the model starts with determination of the number of stages from the best fit slope of logarithm of the incidence on the logarithm of attained age for the unexposed workers for each age-at-start-of-study (essentially birth cohort). This value is approximately 6, indicating the 7 stage model adopted for further analysis of the data set. This is the same value obtained from lung cancer rates in the U.S. population for that set of birth cohorts.

The fit to the model proceeds with and without the multiplicative categorical covariates, calendar year (Cyr) and age-at-start-of-study (Byr). Table E-4 displays the resulting slopes for the calculations, both without covariates and with the Byr covariate for each of the three cases that best fit the data. One case comes from using the next-to-the-last-stage for the ramp exposure pattern, and the other two come from the last stage and the next-to-the-last-stage for the roof exposure pattern. In all these cases the column second from the right hand margin shows a statistically significant improvement of fit for the use of the Byr covariate. This result is offset by the results from calculating AIC and BIC (not given in the table), criteria which penalize the likelihood or deviance differences for adding parameters. For each of the three cases, the AIC values were about the same for both calculations, suggesting the use of the simpler form of the model without covariates. The BIC values clearly favored use of the form of the model without covariates.

Whether or not the covariates are used has little effect on the 95% UCL for the slope. However, some of these slopes define the range of risk of the slopes; so the slightly lower numerical results for the cases using the covariates, Byr, are quoted below. It should be noticed that including only calendar year, Cyr, as a covariate, instead of only Byr, gives rather similar results to using only Byr (numerical results not given here). Including both together in the model also gives similar results.

One of the parameters needed in the analysis is z, the lag from detection to death. The lag used by Garshick *et al*. (1988) to obtain their significant results was 5 years, and this lag did give the most the significant risk slopes for the next-to-the-last-stage models. For the last-stage model a 10-year lag gave the best fit.

The right-hand column in Table D-4 reports the probability that the categorical (stair-step) trend fits the model no better than the loglinear form of trend. In the first two cases, both next-to-the last-stage models, the categorical trend gives a marginally better fit ($p=0.04$) than log-linearity for use of the Byr covariate, while the categorical trend provides no significant improvement of fit without the covariate. In the third case, the roof exposure pattern with the last stage model, the categorical trend provides no significant improvement of fit with or without the covariate. For use of the Byr covariate in each of the three cases, Figures D-4, D-5 and D-6 give a visual impression of the categorical or stair-step trend of relative risk with exposure compared to the log-linear trend of relative risk with exposure assumed in this form of multistage model. A more direct comparison of the trend of observation to prediction is given in Figure D-9. For the case of roof exposure pattern with the next-to-the-last-stage model, this shows the cloud of points for each cell of values that was developed for the analysis.

D.2.3.2 RESULTS FOR THE THREE CASES

Case 1: For the ramp pattern of exposure, the transformation number, i = 6, provides the best explanation of the data with a 5-year lag. This result indicates that the single stage sensitive to diesel exhaust is the next-to-the-last stage (transformation) in the multistage model. With Byr as a covariate the MLE slope is $R_1 = 15 \times 10^{-3}$ (95% CI: 6.1×10^{-3} to 24×10^{-3}) relative hazard per $\mu g/m^3$, corrected for intermittency of 0.33 for the working year. This is a significant slope ($p = 3.0 \times 10^{-3}$). From the life-table calculation, the 95% UCL of the lifetime unit risk for this model is $q_1^* = 3.8 \times 10^{-4}$ $(\mu g/m^3)^{-1}$; the MLE value is $q_1 = 2.4 \times 10^{-4}$ $(\mu g/m^3)^{-1}$.

Case 2: For the roof pattern of exposure, the transformation number, i = 7, in the multistage model provides the best explanation of the data with a 10-year lag. This result indicates that the single stage sensitive to diesel exhaust is the last stage (transformation) in the multistage model. With Byr as a covariate, the MLE slope is $R_1 = 6.6 \times 10^{-3}$ (95% CI: 4.0×10^{-3} to 9.2×10^{-3}) relative hazard per $\mu g/m^3$, corrected for intermittency of 0.33 for the working year. This is a highly significant result ($p = 1.3 \times 10^{-5}$). From the life-table calculation, the 95% UCL of the lifetime unit risk for this model is $q_1^* = 1.5 \times 10^{-4}$ $(\mu g/m^3)^{-1}$; the MLE value is $q_1 = 0.9 \times 10^{-4}$ $(\mu g/m^3)^{-1}$.

Including shop workers in a corresponding analysis produces similar results, with the 95% UCL of unit risk being reduced 15%. In that analysis the exposure concentration for the shop workers is assumed to be the same as for train workers. The rationale for this assumption is that the measurement of concentration in the engine shops above the background of clerks and signalmen is about twice that of the concentration of train workers above that background and that the fraction of shop workers in those engine shops is about one half, which constitutes an unbiased estimate on the basis of no information about what the true fraction is. So if risk is linear in concentration, this procedure gives an unbiased estimate of average exposure concentration for purposes of estimating the risk slope.

Case 3: The form of the model with the next-to-the-last stage sensitive with a 5-year lag, instead of the last stage sensitive, also provides a significant slope, $p = 6 \times 10^{-3}$. With Byr as a covariate, the MLE slope is $R_1 = 5.1 \times 10^{-3}$ (95% CI: 1.8×10^{-3} to 8.5×10^{-3}) relative hazard per $\mu g/m^3$, corrected for intermittency of 0.33 for the working year. From the life-table calculation, the 95% UCL of the lifetime unit risk for this model is $q_1^* = 1.3 \times 10^{-4}$ $(\mu g/m^3)^{-1}$; the MLE value is $q_1 = 0.81 \times 10^{-4}$ $(\mu g/m^3)^{-1}$.

D.3 RESULTS OF ANALYSES USING THE TIME SERIES OF SMRS

The programs available for the Poisson regressions based on categorizations of the individual data did not provide a means of comparing the goodness of fit across the four combinations of assumptions of model and exposure. So the analysis turned to determining the parameters of the models by least-squares fit between the time series of standardized mortality ratios (SMRs) and the predictions of the models. Table D-5 presents the results and indicates the simplifying assumptions used, and Figures D-8 and D-9 show the trends of the risk ratios graphically. The

noisiness of the SMR data relative to predictions is greater in this formulation because of the lack of adjustment for age covariates and the lack of use of individual exposures.

The summed squared residuals (SSRs) of the fits show that the multistage models for both the ramp and the roof exposure patterns fit their respective cases better than the general models using cumulative exposure. The SSRs also show that the roof exposure assumption gives better fits for each of the respective model approaches. Thus the multistage last-stage model with roof exposure pattern appears to have the advantage in fit.

Table D-5 also shows that the unit risk values calculated with this greatly simplified approach compare favorably to the values calculated using the Poisson regression based on categorizing the individual data. The values for the simplified analyses deviate at most 21% from the corresponding values of the more elaborate analyses.

D.4 DISCUSSION

D.4.1 COMPARISON OF THE RESULTS FOR THE FOUR COMBINATIONS OF ASSUMPTIONS

In view of the uncertainties of exposure measure and model selection, the analyses above use four (2 x 2) different combinations of assumptions about measures of exposure and types of models. The four combinations came from the two patterns of exposure, ramp and roof, and the two different model approaches, general and multistage.

Of the 11 general models using either the roof or ramp exposure, the criteria for goodness of fit indicate a preference for the rather rigid model using external (US) rates for lung cancer, controlled for birth cohort, to account for the effects of age and calendar year. However, the set of models for each particular pattern of exposure gives slopes that are well within a two-fold range.

For the preferred general model based on cumulative exposure, the unit risks for the roof pattern are 2.5 times less than those for the ramp pattern. This is consistent with the cumulative exposures being in the ratio of 2.25. The corresponding ratio of unit risks for the 7-stage model is about 3. The weighting of the exposures makes the multistage models more sensitive to the different exposure patterns than the general models.

The predictions of the general model based on cumulative exposure, whether the ramp pattern or the roof pattern, do not appear to be as successful in practice as the 7-stage model. (1) They do not fit the SMR time data as well, as seen in Table D-5 and Figures D-8 and D-9. (2) The relationships between relative risk and cumulative exposure for the general model are not well characterized as linear, as noted in the discussion of each of the models results. Results of including a quadratic as well as a linear term in the analysis also suggest nonlinearity in the general models. (3) The p-values for linear relationship are not nearly as significant although they are still quite significant, also as noted above. In addition, the 7-stage model appears

preferable for the important theoretical reason of taking age into account in a specific way derived for the multistage cancer process.

The multistage model obtains approximately 3-fold lower risks than the general model, for each pattern of exposure. The multistage model that best fits the data, assuming the roof pattern of exposure, is a 7-stage model with the 7th transformation sensitive to diesel exhaust. The unit risk obtained is $q_1^* = 1.3 \times 10^{-4}$ (lifetime-$\mu g/m^3)^{-1}$, and this result is highly statistically significant ($p = 2 \times 10^{-4}$). The categorical trend for this model is not significantly different from the log-linear trend ($p = 0.45$). This fit requires a 10-year lag between the last transformation and death, compared to a 5-year lag for the models in the other analyses. This last-stage model with roof exposure pattern shows the clearest response to diesel exhaust of any of the combinations.

The 10-year lag for the last-stage model corresponds approximately to the 5-year lag of the next-to-last stage model because that model has an expected value of 5 years from each exposure until transformation, added to the 5-year lag from transformation until death. Thus this 10-year lag is quite plausible and is not at all the same kind of lag as the short (few-year) lag from clinical detection to death from lung cancer.

For the roof exposure pattern, the risk slopes for the next-to-last-stage model are quite close to those for the last-stage models and the fit is nearly as good.

For both the roof and ramp pattern, the categorical trend for the next-to-the-last-stage models exhibits a downturn of relative risk with increasing exposure at the highest exposures, but this downturn is not as pronounced as for the general models. The less successful performance of the general models based on cumulative exposure can be understood on the basis of the multistage concept. The use of cumulative exposure in the general models implies that, though they have a more flexible age structure than the strict multistage model, they are essentially two-stage models with the first stage active. This is verified by specializing the multistage integral above to this case. As early-stage models, they cannot reflect a decline in exposure concentration with a decline in incidence, as the late-stage models do.

Each of the analyses use a straight line to approximate the responses of a large and complex cloud of points, which are only very schematically represented by the categorical plots in Figs. D-2 through D-6. The apparent falloff in incidence of the highest exposure category, shown in most of those figures, also occurred for some of the analyses of arsenic (DHS, 1990, pp. 11-1 to 11-47). As in the third combination, arsenic appeared to have a late-stage transformation when analyzed by the multistage model (Brown and Chu, 1983). Cook *et al*.(1969) explored reasons for the falloff of cancer rates with high exposures. This falloff occurs with age for lung cancer in the general population, in which age may be considered a surrogate for exposure. For further comparison, cigarette smoking appeared to cause both a late- and an early-stage transformation when analyzed by a multistage model (Doll, 1971; Day and Brown, 1980; Brown and Chu, 1987). The present results appear to be qualitatively consistent with a combination of next-to-last-stage and last-stage forms of the model.

D.4.2 COMPARISON TO OTHER WORK ON THE COHORT

Comparison of these results to those of others who have analyzed the original data tends to emphasize uncertainties and potential pitfalls in such analyses. The analysis of Garshick *et al*. (1988) and the first set of analyses of Crump *et al*. (1991) and the Garshick (1991) letter all assume that exposure started at the beginning of follow-up, in 1959, and include shop workers and all 22 years of follow-up in the analysis.

These analyses all obtained risks for categories of increasing duration of exposure, but not slopes. The resulting trends appeared to differ, as discussed in Section 7.3.4.3.1, but slopes fitted retrospectively to those results are of the same magnitude as the results in this appendix. Section 7.3, Table 7-10 presents a range of results for slopes from analyses based on duration of exposure as well as the results of this appendix.

The present analyses use an exposure pattern going back to 1945 and exclude especially uncertain data. The findings for general models suggest that neither the model using birth cohort and calendar year independently, essentially as in Garshick *et al*. (1988) nor the model using attained age and calendar year independently, as in Crump *et al*. (1991) and Crump (1995, 1996a), provide as good a fit as using the last-stage models.

ETS-adjusted RSP is one of the five measures of exposure concentration used by Crump *et al*. (1991) and Crump (1994, 1995, 1996a, 1996b) that do not subtract the unexposed background from the exposed concentration. Because the background concentration for the unexposed group is not subtracted from the exposure concentrations, the analyses treat the unexposed group as the lowest exposed category, the individuals of which are then subject to the paradox of having increasing cumulative exposures over time, as pointed out in the Garshick (1991) letter. In an analysis that accumulated exposure for a ramp pattern with background included, Crump *et al*. (1991) and Crump (1994) used one of the internal models, the one multiplicative in categories of attained age and calendar year independently, with an inaccurate allocation of cancer incidence rates in the initial cross-tabulation. With a corrected cross-tabulation, Crump (1995, 1996a) used that model and the simplest of the models with external standardization to calculate slopes, finding no statistically significant positive slopes for 80 different cases. These cases included separate controls for job category and different selection of data -- with and without the last four years of the follow-up and with and without shop workers, hostlers and signalmen.

Part of the difference between those results and the current results for general models is due to differences in the choice of models. The second analysis, the general model with the ramp exposure pattern, contains the cases closest to those of Crump *et al*. (1991). Subtracting background appears in itself to result in substantially increased statistical significance from the analyses. In contrast to Crump (1995,1996a), the slopes produced in all the current analyses are significant.

D.4.3 UNCERTAINTIES IN THE ANALYSES

In the present work the choices of data were generally made on the basis of plausibility in the judgment of those responsible for the reanalysis after consultation with experts, for example at the January 1996 workshop on diesel exhaust risk assessment. Other uncertain choices were made on the basis of numerical criteria in the calculations. The preference for a model, if necessary, generally used a criterion for goodness of fit. Exposure category boundaries were chosen to equalize cancer deaths in the exposed categories, initially in order to equalize the variances of the estimates of relative hazard across categories so that visual and sequential comparisons would be most accurately presented. This choice became convenient for providing a consistent, reasonable approach among a myriad of possibilities. The related choice of the number of exposure categories was based mainly based on obtaining a smooth visual representation of the trend with the use of several trials although several figures show results for many categories as an example. The choice of time lag from carcinogenesis to death was made at 5 or 10 years so as to obtain the best fit and to obtain statistical significance, as in Garshick *et al*. (1988).

The above comparison of the present results to those of others who have analyzed the original data serves to characterize uncertainty in these analyses. The work of Crump (1995, 1996a, 1996b) has been especially useful in this regard. Different choices of model structure, category boundaries and selection of data can lead to non-significant results or results of questionable significance, at least for the results that are not very statistically significant. Preliminary analyses with a cumulative exposure model for the ramp pattern with background included, as in the Crump analyses, gave marginally significant results for the slope. Then investigation showed that a number of ways of changing particular assumptions gave results that were not statistically significant. Including the shop workers resulted in nonsignificance; including the last four years resulted in nonsignificance, even when using a spline function or yearly variable to correct the rates during that time; several ways of changing exposure category boundaries resulted in nonsignificance.

These results show that not subtracting background results in p-values that vary rather widely around 0.05; so the statistical significance of the slopes is not very robust with respect to several changes in assumptions. In the analysis using the last stage model and the roof exposure pattern with background subtracted, on the other hand, changes such as including shop workers results in little change of significance in the slope of risk versus exposure. This analysis is much more robust with respect to changing such assumptions.

D.5 CONCLUSION

From four combinations of assumptions about exposure characterization and types of models the overall range of 95% UCL for unit risk includes values for the preferred models between 1.3×10^{-4} and 24×10^{-4} for lifetime exposure to 1 $\mu g/m^3$. These values resulted from an investigation to determine statistically significant slopes within the framework of the most plausible combinations of assumptions about data and models. All of these results are clearly statistically significant ($p<0.01$). These results are in contrast to the Crump re-analyses (1995, 1996a,

1996b), which found no statistically significant results, with a conclusion of no convincing evidence of an association between risk of lung cancer and duration of exposure to diesel exhaust or quantitative measures of exposure to diesel exhaust.

The value for the low end of the range of unit risk, 1.3×10^{-4} $(\mu g/m^3)^{-1}$, appears to provide the best overall fit of the data. The lower end of the range is the rounded value for both forms of multistage model using the roof exposure pattern for the data of the Garshick *et al*. (1988) cohort study of U.S. railroad workers.

Table D-1. LIFE TABLE TO ESTIMATE CALIFORNIA LUNG CANCER RISK BY AGE CATEGORY

Uses (1) Department of Finance population projection for July 1, 1991; (2) deaths by age... Table 5-6, Vital Statistics of California, DHS 1991; (3) five-year cancer incidence, Table XIV -5, Cancer Incidence and Mortality in California by Detailed Race/Ethnicity, 1988-1992, DHS 1995

	m(i)= (i)/d(i)	p(i)=1-q(i)	C(i)= C(i-1)*p(i-1)	l(i)	pl(i)= l(i)/m(i)*q(i)	u(i)= pl(i)*C(i)	s(i) s(i-1)+u(i-1)		THE RAW DATA n(i)	d(i)
age interval (i,i+4)	1991 California annual death rate per [10^5] in age interval (i,i+4)	P (survival to age i+4 given survival to age I)	cumulative P (survival to age I)	1988-92 California annual lung cancer death rate per [10^5] in (i,i+4)	P (lung cancer death, given survival to age I)	unconditional P (lung cancer death in (i, i+4))	cumulative P (lung cancer death by age i+4)		California population projection 1991	California all deaths 1991
0-4	208.5	0.990	1.000	0.000	0.00E+01	0.00E+01			2622793	5468
5-9	19.8	0.999	0.990	0.000	0.00E+01	0.00E+01	0.000000		2272842	450
10-14	25.1	0.999	0.989	0.000	0.00E+01	0.00E+01	0.000000		2072016	520
15-19	95.7	0.995	0.987	0.000	0.00E+01	0.00E+01	0.000000		2031176	1943
20-24	108.9	0.995	0.983	0.100	4.99E-05	4.90E-05	0.000005		2542302	2769
25-29	116.0	0.994	0.977	0.200	9.97E-05	9.75E-05	0.000015		2829444	3282
30-34	152.1	0.992	0.972	0.700	3.49E-04	3.39E-04	0.000049		2922466	4444
35-39	205.9	0.990	0.964	2.600	1.29E-03	1.25E-03	0.000173		2587937	5328
40-44	262.4	0.987	0.954	8.200	4.07E-03	3.89E-03	0.000562		2282160	5989
45-49	367.1	0.982	0.942	25.000	1.24E-02	1.17E-02	0.001729		1667721	6122
50-54	533.0	0.974	0.925	56.300	2.78E-02	2.57E-02	0.004298		1337454	7129
55-59	837.2	0.959	0.901	103.000	5.04E-02	4.54E-02	0.008840		1140360	9547
60-64	1283.6	0.938	0.864	167.000	8.09E-02	6.98E-02	0.015825		1110088	14249
65-69	1919.7	0.908	0.810	231.000	1.10E-01	8.92E-02	0.024745	← target	1062044	20388
70-74	2927.8	0.864	0.736	295.000	1.37E-01	1.01E-01	0.034842		841694	24643

Formulas for this life table from Health Effects of Cadmium, California Department of Health Services (DHS), 1986.

Table D-2. Risk Trends For Roof Exposure.

Background subtracted from ETS-adjusted RSP exposure concentration. Excludes shopworkers and last 4 study yrs. Cancer deaths equalized in 6 exposure categories.
Analysis 1: General Models

Control for age & year *(a)*	Slope MLE *(b)*	Std. err *(c)*	95% LCL *(d)*	95% UCL *(e)*	Linear Exposure Model p(t-test) *(f)*	Deviance *(g)*	df *(h)* n= 461 *(i)*	AIC *(j)*	BIC *(k)*	Categorical Exposure Model Deviance	df *(h)* n= 461 *(i)*	p(fit diff.) *(l)*
categorical:												
Nrate	8.8E-04	3.3E-04	3.3E-04	1.4E-03	4.2E-02	577.9	459	-291.0	-295.1	546.4	455	2.4E-05
						0.0010 = p chisq \	(m)		(max)			(S)
Nrate + Byr	1.2E-03	3.4E-04	6.0E-04	1.7E-03	3.7E-03	559.5	455	-285.8	-298.2	533.9	451	3.8E-04 (S)
Aage + Cyr	1.0E-03	3.7E-04	4.1E-04	1.6E-03	3.0E-02	583.6	452	-300.8	-319.4	563.3	448	4.3E-03 (S)
Byr + Cyr	1.3E-03	3.8E-04	7.2E-04	2.0E-03	2.0E-03	572.3	451	-296.1	-316.8	559.6	447	1.3E-01 (S)
Byr + Cyr + Aage	1.2E-03	3.8E-04	6.0E-04	1.8E-03	6.5E-03	543.6	448	-284.8 (max)	-311.7	527.7	444	3.2E-02 (S)
ByrCyr	1.2E-03	3.8E-04	5.9E-04	1.8E-03	6.8E-03	543.8	435	-297.9	-351.6	526.0	431	1.3E-02 (S)
AageCyr	1.1E-03	3.7E-04	4.8E-04	1.7E-03	1.8E-02	559.4	443	-297.7	-334.9	540.7	439	9.0E-03 (S)
continuous												
aage + aage^2	1.5E-03	3.4E-04	8.9E-04	2.0E-03	1.3E-04	607.0	457	-307.5	-315.7	583.7	453	1.1E-03 (S)
aage+ ^2 + cyr+ ^2	1.0E-03	3.7E-04	4.0E-04	1.6E-03	3.5E-02	594.7	455	-303.3	-315.7	573.0	451	2.3E-03 (S)
byr+ ^2 + cyr+ ^2	1.2E-03	3.8E-04	6.1E-04	1.8E-03	6.0E-03	577.8	455	-294.9	-307.3	563.1	451	5.3E-02 (S)
+ byr*cyr	1.1E-03	3.7E-04	4.9E-04	1.7E-03	1.7E-02	560.3	454	-287.1	-301.6	537.7	450	1.6E-03 (S)

Table D-3. RISK TRENDS FOR RAMP EXPOSURE.

Excludes shopworkers and last 4 study yrs. Cancer deaths equalized in 4 exposure categories.
Analysis 2: General Models

Control for age & year (a)	Slope MLE (b)	Std. err (c)	95% LCL (d)	95% UCL (e)	Linear Exposure Model p(t-test) (f)	Deviance (g)	df (h) n=352 (i)	AIC (j)	BIC (k)	Categorical Exposure Model Deviance	df (h) n=352 (i)	p(fit diff.) (l)
categorical:												
Nrate	2.0E-03	9.1E-04	5.6E-04	3.5E-03	1.2E-01	455.178	350	-229.6	-233.5	442.4	348	1.7E-02
						0.0012 =p chisq \ (m)			(max)			(S)
Nrate + Byr	2.9E-03	9.4E-04	1.3E-03	4.4E-03	1.2E-02	437.108	346	-224.6	-236.1	427.9	344	9.9E-02 (S)
Aage + Cyr	2.5E-03	1.0E-03	8.2E-04	4.2E-03	7.5E-02	460.675	343	-239.3	-256.7	454.9	341	5.5E-01
Byr + Cyr	3.5E-03	1.0E-03	1.8E-03	5.2E-03	4.7E-03	449.302	342	-234.7	-254.0	448.9	340	8.3E-00
Byr + Cyr + Aage	3.1E-03	1.0E-03	1.4E-03	4.8E-03	1.6E-02	420.775	339	-223.4 (max)	-248.5	417.3	337	1.8E-00
ByrCyr	3.1E-03	1.0E-03	1.4E-03	4.8E-03	1.7E-02	421.035	326	-236.5	-286.7	416.6	324	1.1E-00
AageCyr	2.7E-03	1.0E-03	1.0E-03	4.4E-03	4.4E-02	436.461	334	-236.2	-271.0	431.3	332	7.7E-01
continuous:												
aage + aage^2	3.7E-03	9.3E-04	2.2E-03	5.3E-03	4.2E-04	484.702	348	-246.4	-254.1	479.0	346	5.7E-01
aage+ ^2 + cyr+ ^2	2.5E-03	1.0E-03	7.8E-04	4.2E-03	8.5E-02	471.741	346	-241.9	-253.5	463.5	344	1.6E-01 (S)
byr+ ^2 + cyr+ ^2	3.1E-03	1.0E-03	1.4E-03	4.9E-03	1.4E-02	454.822	346	-233.4	-245.0	450.4	344	1.1E-00
+ byr*cyr	2.7E-03	1.0E-03	1.0E-03	4.4E-03	4.5E-02	437.474	345	-225.7	-239.3	428.2	343	9.7E-02 (S)

Table D-3. RISK TRENDS FOR RAMP EXPOSURE.

Footnotes for TABLES D-2 and D-3

a Gives symbolic description of covariate structure of the loglinear models
Plus sign (+) indicates the variables enter the model independently.
Juxtaposed variable names indicate that the variables enter the model interactively.
Nrate = the US rate for lung cancer
Byr = category of birth year
Cyr = category of calendar year
Aage = category of attained age
aage = attained age as a continuous variable
cyr = calendar year as a continuous variable
byr = birth year as a continuous variable

b Maximum likelihood estimate (MLE) of the slope (units of working yr-$\mu g/m^3$).

c Standard error of the slope estimate (units of working yr-$\mu g/m^3$).

d 95% lower confidence limit for the slope estimate
(units of working yr-$\mu g/m^3$).

e 95% upper confidence limit for the slope estimate
(units of working yr-$\mu g/m^3$).

f Numerical result of significance test for the slope estimate.

g Deviance for the MLE. Sum of contributions based on Poisson distribution:

$D_i = 2 \{ c_i \ln(c_i / P_i\lambda_i) - (c_i - P_i\lambda_i) \}$ = deviance contribution where
c_i = number of cancer deaths observed in cell i of cross-tabulation,
P_i = number of person years observed in cell i of cross-tabulation,
λ_i = rate function, evaluated by model prediction for cell i.

h Degrees of freedom

i Number of data points (non-empty cells) in the Poisson Regression.

j Modified Akaike's information criterion for selecting the best fitting model.

k Modified Bayesian information criterion for selecting the best fitting model.

l Significance level of improvement of fit in going from slope to categorical model for exposure trend. Uses chi-squared test.

m Significance level of improvement of fit due to enlarging the model based on national rates to include multiplicative categorical variables for effect of the birth cohort of the railroad worker. Uses chi-squared test.

Table D-4. Results for Slope (R1) of Hazard with Concentration for Multistage Models. Garshick *et al*. (1988) Cohort

	R1			probability (> 0.05 ?)		
	0.33*R1					
	MLE	**LCL**	**UCL**	slope not > 0	fit with Byr no better	steps fit no better
Ramp Exposure Pattern						
6/7-stage model with covariates:						
Wtexp+Ln(aage)	1.9E-01	1.1E-01	2.8E-01	1.2E-03	-	0.11
Wtexp+Ln(aage) +Byr	1.5E-01	6.1E-02	2.4E-01	3.0E-02	0.024	0.04
Roof Exposure Pattern						
6/7-stage model with covariates:						
Wtexp+Ln(aage)	6.9E-02	3.7E-02	1.0E-01	2.4E-03	-	0.25
Wtexp+Ln(aage) +Byr	5.1E-02	1.8E-02	8.5E-02	6.0E-02	0.027	0.04
Roof Exposure Pattern						
7/7-stage model with covariates:						
Wtexp+Ln(aage)	6.6E-02	4.0E-02	9.2E-02	1.3E-04	-	0.31
Wtexp+Ln(aage) +Byr	5.7E-02	3.0E-02	8.3E-02	2.0E-03	0.033	0.45

TABLE D-5. SUMMED SQUARED RESIDUALS FOR FITTING THE PREFERRED MODELS TO THE SMRS IN TIME [a]

ASSUMPTIONS	General model using cumulative exposure [b]		Armitage-Doll 7-stage model [c]	
Exposure pattern	SSR	(Q_1 (smr)/Q_1)	SSR	(R_1 (smr)/R_1)
Roof	1.09	(1.09)	1.02[e] 1.06[d]	0.91[e] 1.34[d]
Ramp	1.12	(1.21)	1.09[d]	1.24[d]

a Summed squared residuals (SSR) are for the first 18 years of follow-up. Also shown, in parentheses, are the ratios of the values of risk coefficient obtained in this least-squares fit based on SMRs to the preferred MLE risk coefficient obtained in the full analysis of the individual data. Lag from carcinogenesis to death was assumed to be 5 years except in one case, indicated to the contrary.

b Assumes incidence is linear in cumulative exposure, without regard to age. See Figure D-8 for the prediction compared to the SMRs.

c Total number of transformations (7) and the selection of sensitive stage were obtained from the full analysis of the individual data. This analysis assumed that the entire cohort was 37.5 years of age in 1945, reflecting the average age. Uses discrete form of the Armitage-Doll integral defined in the text. See Figure D-9 for the prediction compared to the SMRs.

d Next-to-last (6th) transformation acted on by diesel exhaust.

e Last (7th) transformation acted on by diesel exhaust. Lag from carcinogenesis to death is increased to 10 years for improved fit.

Figure D-1: Exposure Patterns Assumed For Railroad Workers

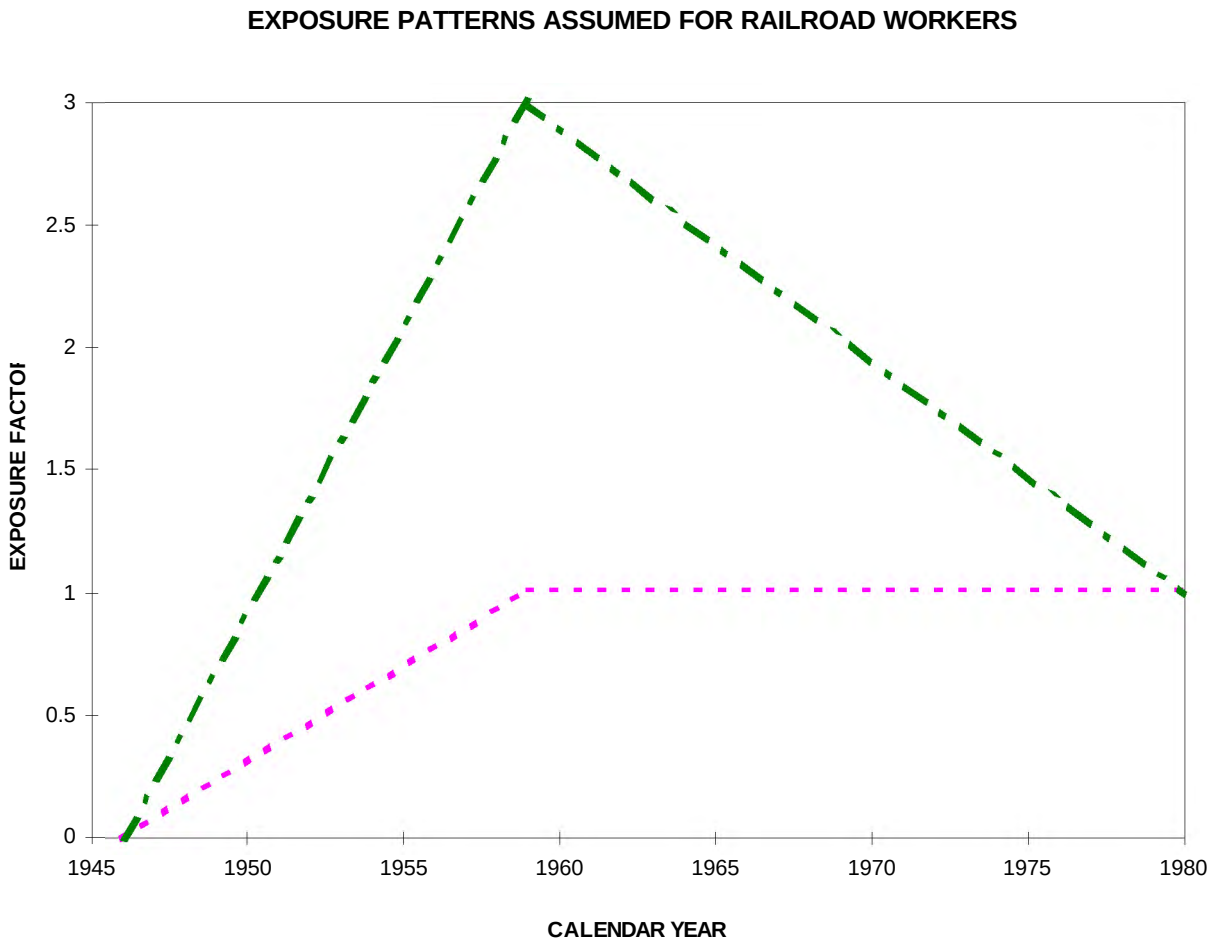


Figure D-2: Fitted Relative Risk, Standardized By US Rates Controlled For Birth Cohort. Roof Exposure; General Model; Background Subtracted.

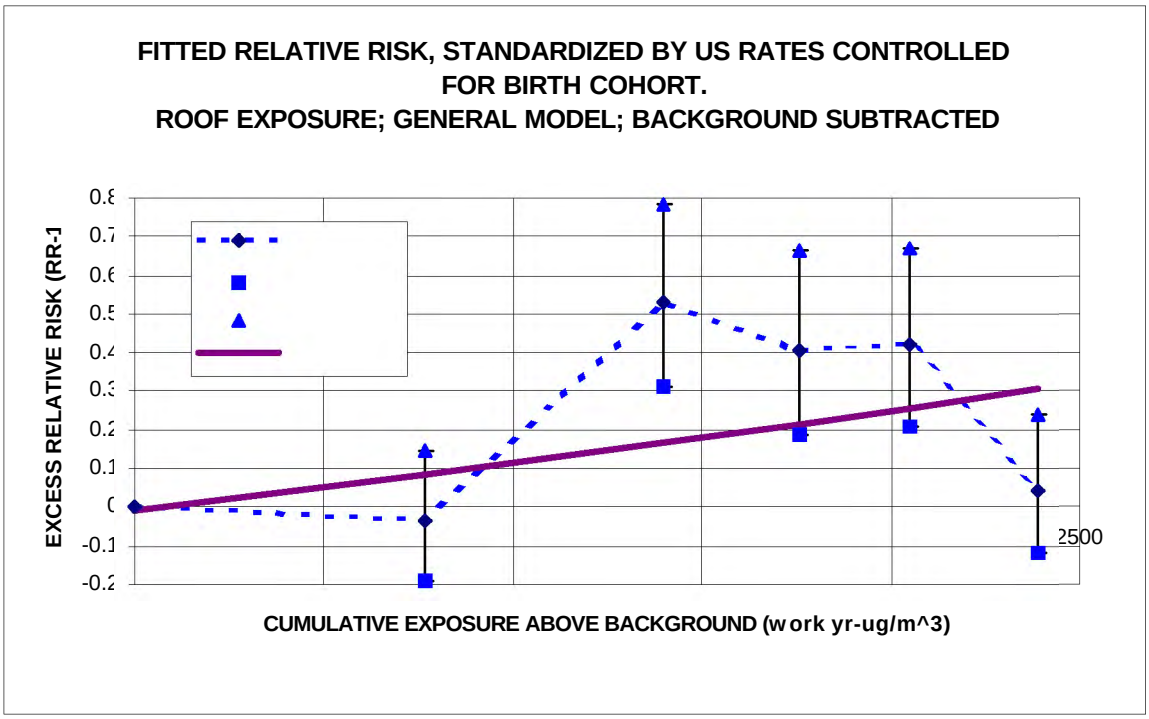


Figure D-3: Fitted Relative Risk, Standardized By US Rates Controlled For Birth Cohort. Ramp Exposure; General Model; Background Subtracted.

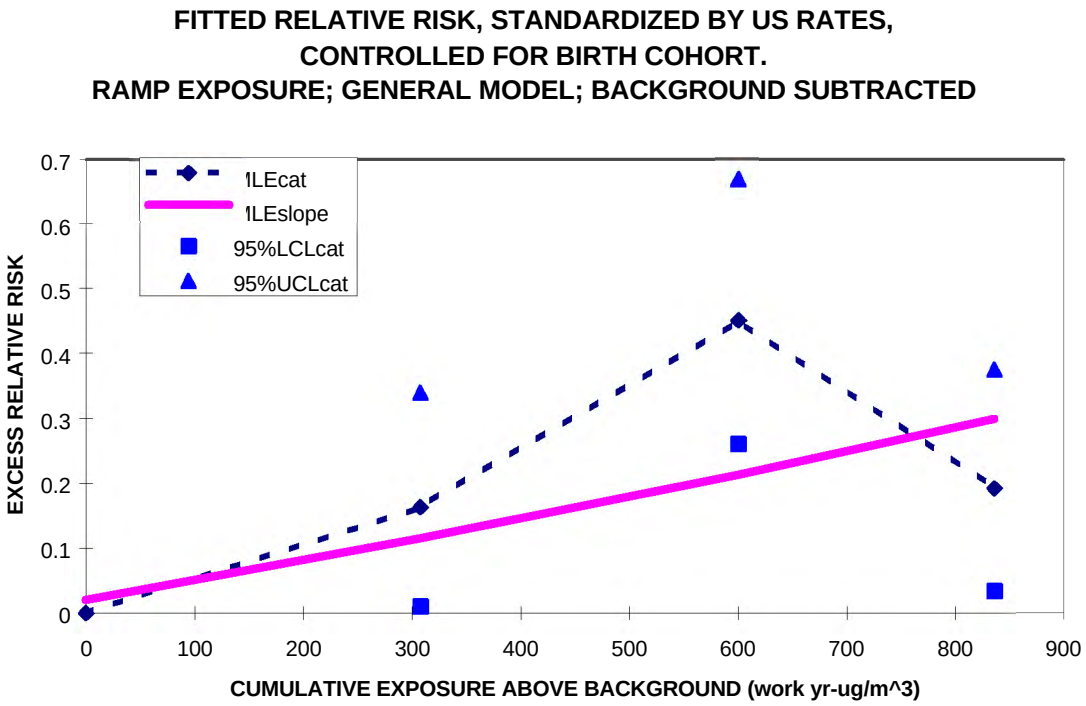


Figure D-4: Relative Risk: 7-Stage Armitage-Doll Model Controlled For Birth Cohort. Next-To-Last Stage Sensitive To Diesel Exhaust; Ramp Exposure Pattern.

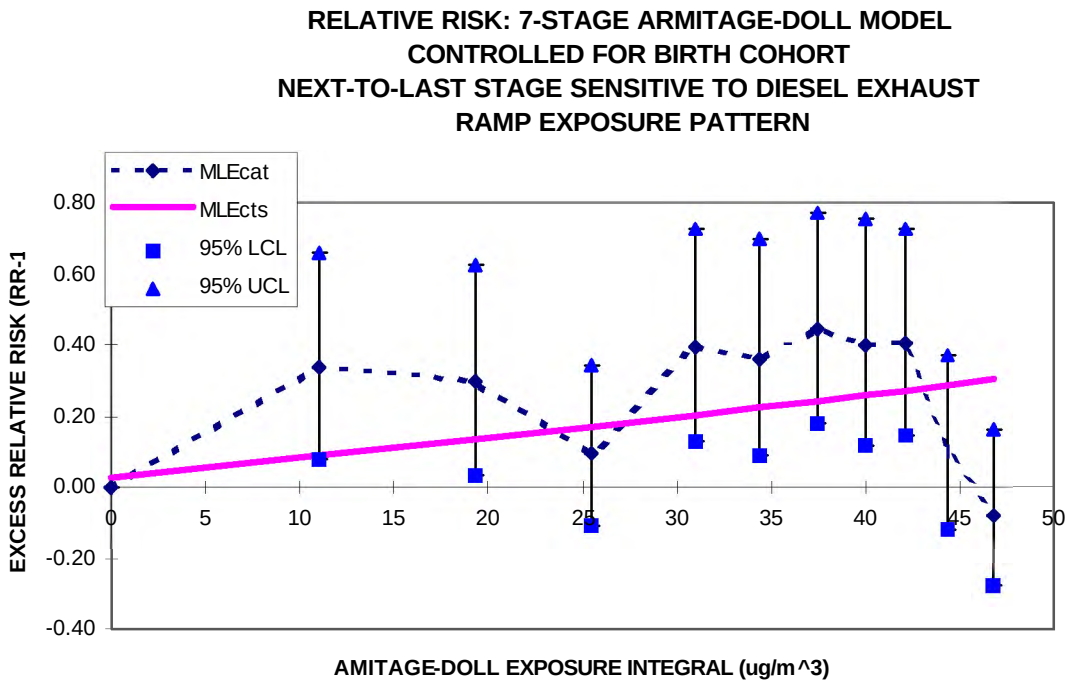


Figure D-5: Relative Risk: 7-Stage Armitage-Doll Model Controlled For Birth Cohort. Last Stage Sensitive To Diesel Exhaust; Roof Exposure Pattern.

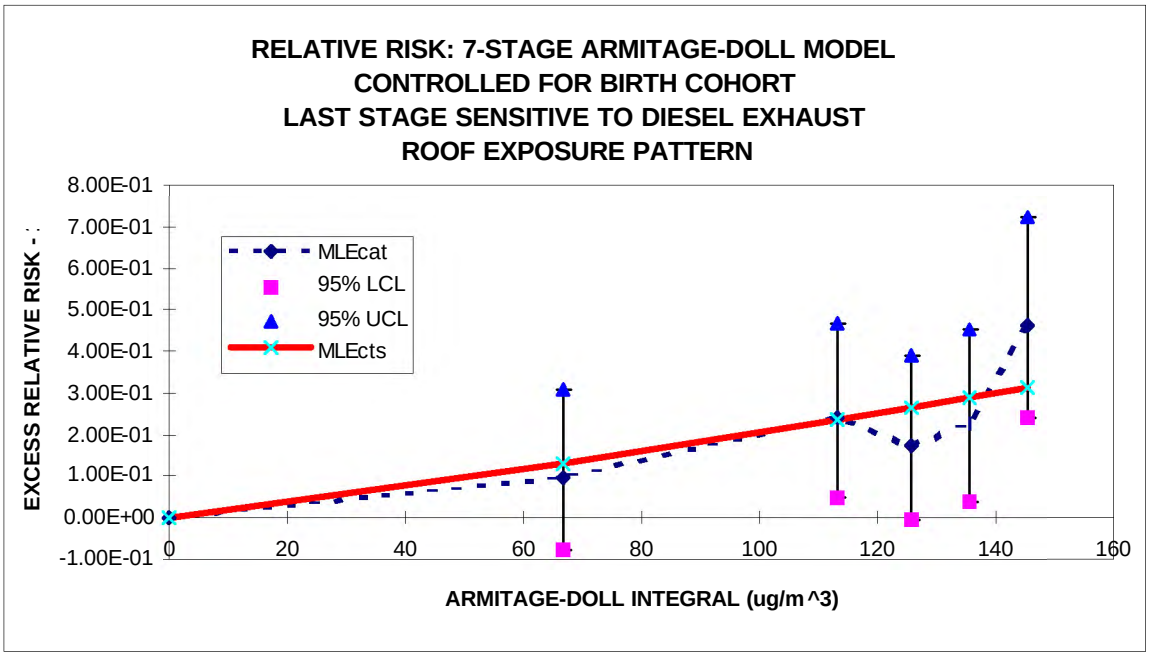


Figure D-6: Relative Risk: 7-Stage Armitage-Doll Model Controlled For Birth Cohort. Next-to-Last Stage Sensitive To Diesel Exhaust; Roof Exposure Pattern.

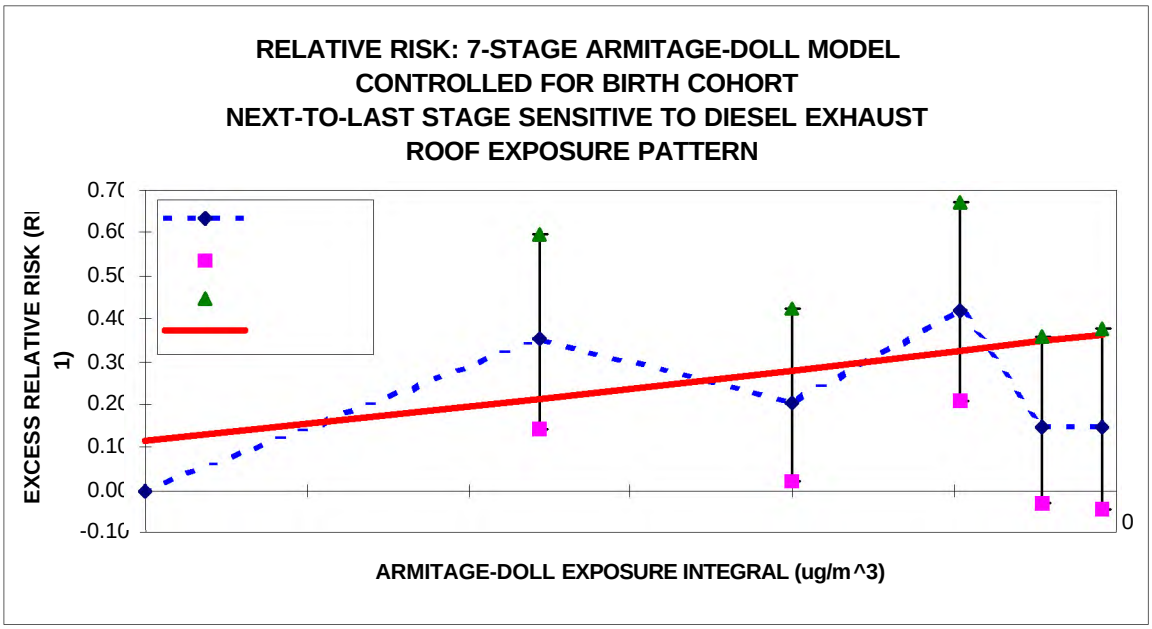
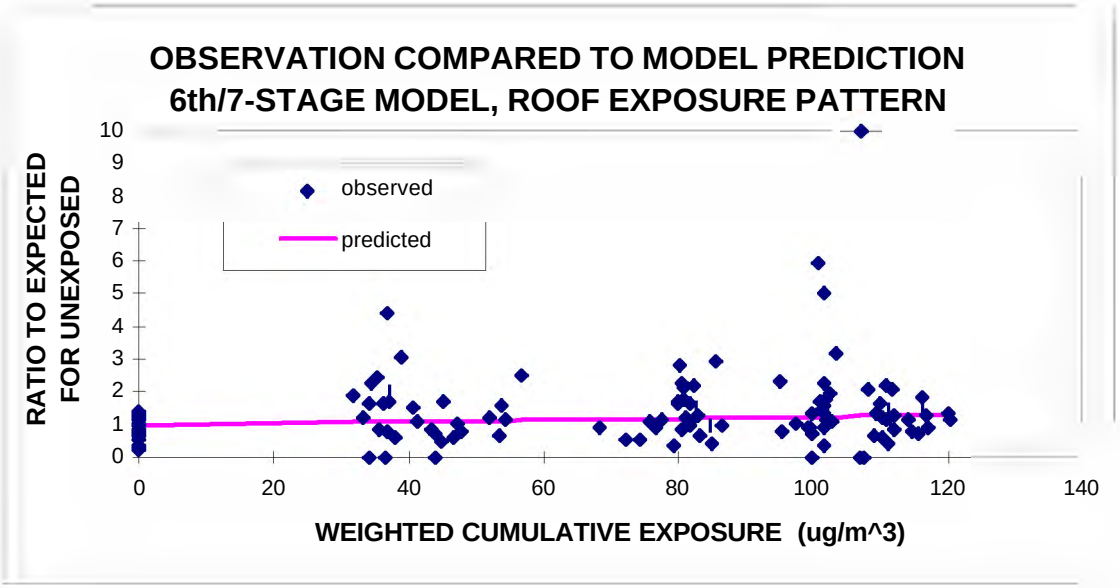


Figure D-7: Observation Compared To Model Prediction. 6th/7-Stage Model, Roof Exposure Pattern.



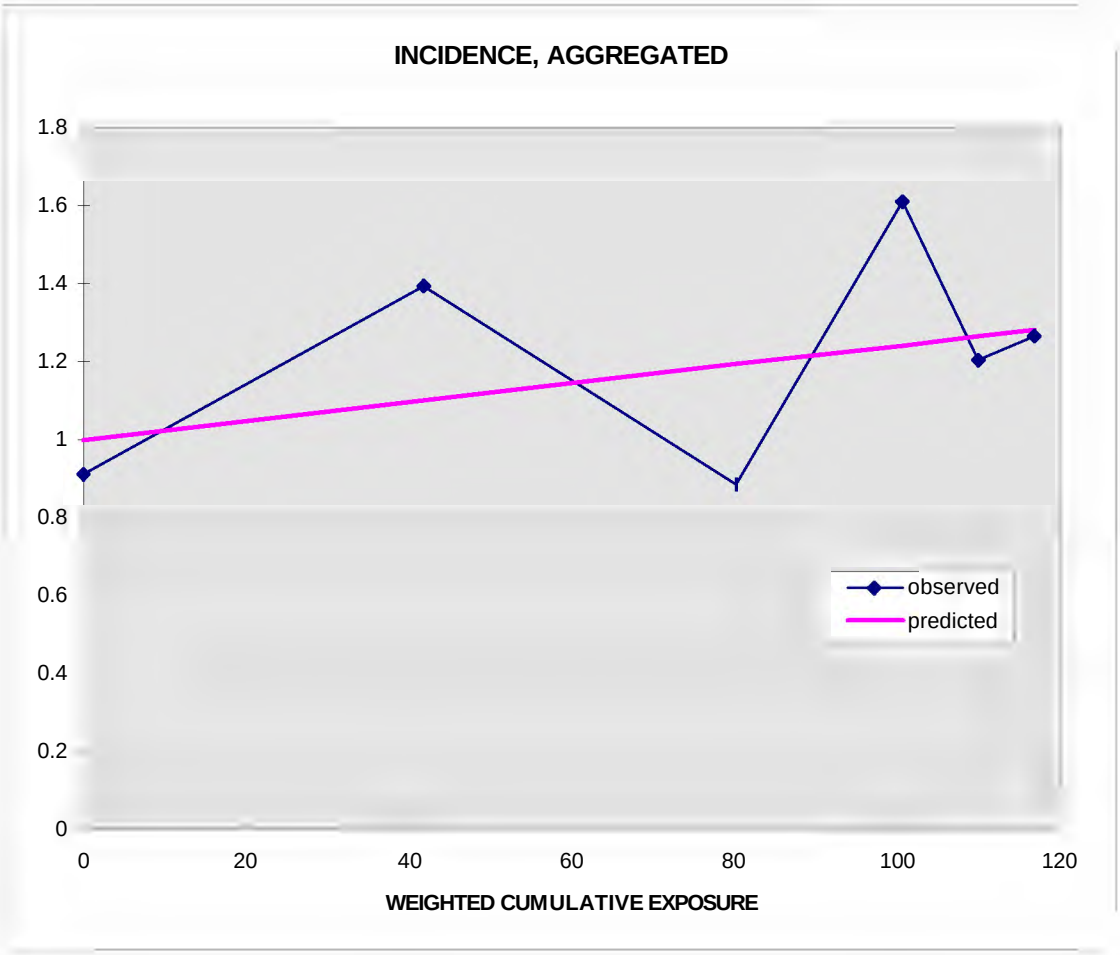


Figure D-8: Predicted Relative Risk Ratios From General Models With Cumulative Exposure Compared To Standardized Mortality Ratios (SMRs)

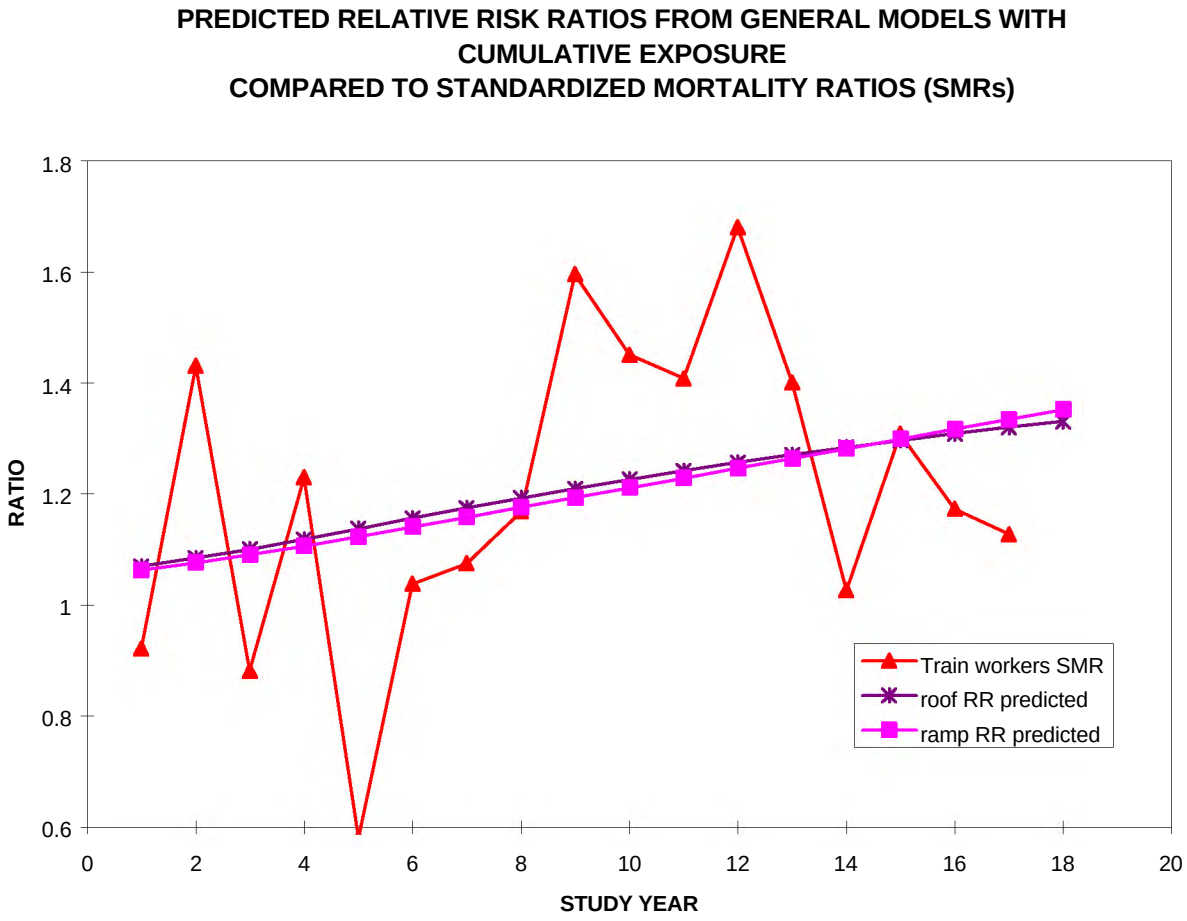
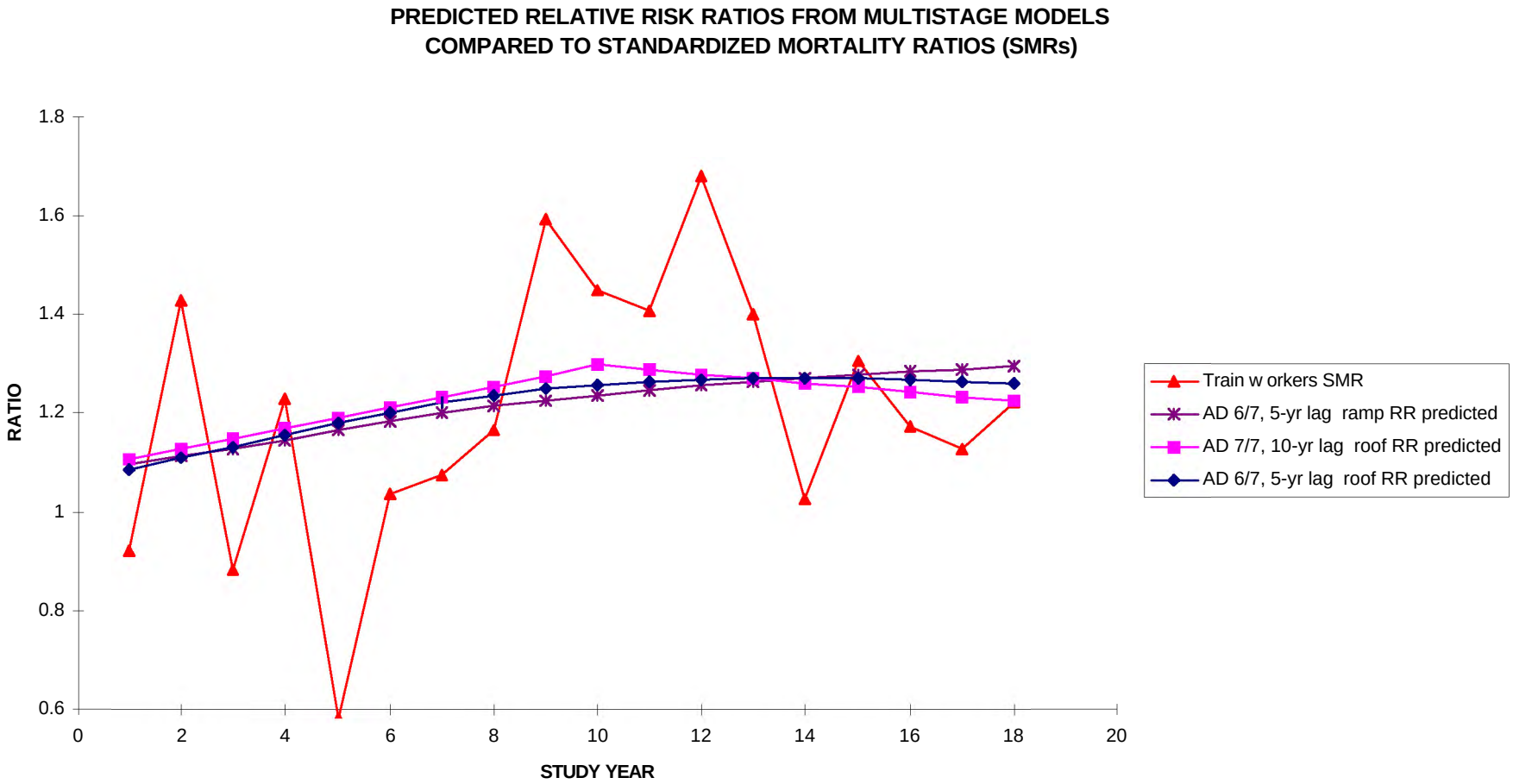


Figure D-9: Predicted Relative Risk Ratios From Multistage Models Compared To Standardized Mortality Ratios (SMRs)



APPENDIX E

DIFFERENCES IN APPROACHES OF DR. STANLEY DAWSON[1] AND DR. KENNY CRUMP[2] TO ANALYZING THE GARSHICK *ET AL.* (1988) COHORT

The Garshick *et al.* (1988) cohort study has been identified as being among the most comprehensive and extensive epidemiological data sets with regards to the carcinogenic effects of diesel exhaust exposure. Even so, due to limitations in the data set, and epidemiological studies in general, a number of questions have been raised as to the application of this data set in the quantitative risk assessment of diesel exhaust.

One of the key issues with regard to the cohort is whether or not a dose-response was obtained. The original publication indicated that among this cohort there was a small but significantly elevated risk for lung cancer. In December 1994, U.S.EPA released a comprehensive health review of diesel exhaust. This draft document included the Crump *et al.* (1991) reanalysis of the dose-response of Garshick *et al.* (1988) cohort study. Subsequently, Dr. Dawson of OEHHA submitted to U.S.EPA a critique of that reanalysis, to which Dr. Crump responded. In January, 1996, the Health Effects Institute (HEI) organized a joint workshop with the California Air Resources Board (CARB), U.S.EPA, the World Health Organization (WHO), the National Institute for Occupational Safety and Health (NIOSH), and OEHHA to bring together recognized experts and interested parties to help clarify and resolve differences in analyzing and interpreting the epidemiological data on diesel exhaust, particularly the Garshick *et al.* (1988) cohort study. Since that time, Dr. Dawson and Dr. Crump have continued a scientific exchange regarding their assessments of the Garshick data. While Dr. Dawson continues to differ with Dr. Crump in the scientific interpretation of the Garshick data, the differences in assumptions, approaches and results now appear to be clarified and understood by Dr. Dawson and Dr. Crump.

The purpose of this section is to summarize the basis for the differences in findings and interpretations of the Garshick *et al.* (1988) cohort study. The information summarized here reflects the scientific record until July 1996. There are five major areas of discussion: choice of model, data selection, measure of exposure, exposure category boundaries and the allocation of cancer deaths across exposure groups, and interpretation of the trend of risk of lung cancer with increasing diesel exhaust exposure. These issues are summarized below.

1. Controlling for Age in the Model

One key difference between Drs. Dawson and Crump in their approach to analyzing these data is how they controlled internally for attained age in the models that they fit to the data. Dr. Crump (1995, 1996a) used both internal and external controls for age and calendar year. For internal controls, Dr. Crump used attained age and calendar year as the independent multiplicative variables in his model. Analyses conducted with this model proceed with three time-dependent

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[1] Dr. Stanley V. Dawson, Office of Environmental Health Hazard Assessment, Air Toxicology and Epidemiology Section, 2151 Berkeley Way, Annex 11, Berkeley, CA 94704; E-mail: sdawson@berkeley.cahwnet.gov.
[2] Dr. Kenny S. Crump, ICF-Kaiser, 602 East Georgia Avenue, Ruston, LA 71270; E-mail: kcrump@lamerica.net.

variables -- attained age, calendar year, and cumulative exposure. On the other hand, Dr. Dawson conducted his analyses with one time-independent variable, age at start of study, and two time-dependent variables, calendar year and cumulative exposure. While he (1995) previously employed the values independently as internal controls, Dr. Dawson (1996a, 1996b) later used an interactive model which, he claimed, fully characterizes the age effect without using attained age explicitly. This model used the interaction of starting age (in this case, age in 1959) and calendar year as a matrix of explanatory variables.

According to Dr. Dawson, the manner in which age is controlled for is an important issue because attained age is highly correlated with the other two time-dependent variables in the analysis (i.e., calendar year and cumulative years of exposure) and this correlation can contribute to uncertainty. Dr. Crump has stated that controlling for attained age and calendar year independently is more appropriate than controlling with the interaction because the independent controls resulted in a smaller deviance with fewer parameters in his analysis (1996a). In contrast, Dr. Dawson (1995b) claimed that there appears to be instability in the results of the independent model as suggested by high values of the correlation coefficient among parameters, and that use of the additional time variable (attained age) masked the effect of exposure. However, Crump (1996b) pointed out that whereas Dr. Dawson's concern regarding the instability of the "independent model" stemmed from estimation of "so many parameters" (Dawson, 1995), his interactive model contains even more parameters. Dr. Dawson conceded that Crump's use of attained age and calendar year independently does appear to have an advantage in that it has 12 fewer parameters and about the same deviance while still giving nearly the same exposure trend as using the interactive model. However, Dr. Dawson claimed that the interactive model has the advantage of describing the background rates most accurately, as long as the categorical variables used (in this case age and time categories) both span the same matrix dimensions, although this condition is not met by the specific models being discussed.

At the January 1996 Workshop (Workshop on Epidemiology-based Quantitative Risk Assessment/Analysis of the Garshick *et al.* 1988 Cohort Study), it was suggested that a biologically-based model (such as Armitage-Doll or Moolgavkar) model be utilized in order to further explore the exposure-time-response relationship. Development of such a model is as described in Appendix E of the March 1997 draft document. This analysis was not provided to Dr. Crump for his review prior to publicly issuing Appendix E described in the March 1997 draft document. In addition, to the analyses discussed above, Appendix E of the March 1997 draft included analyses based on attained age.

2. Data Selection

A. Exposure estimates for shop workers

In the 1991 letter from Dr. Garshick to U.S.EPA, Garshick discussed the limitations of the exposure estimates from Woskie *et al.* (1988a) for the shop workers. Specifically, he noted that within the shop worker exposure group there were some shop workers who worked in diesel repair shops with high diesel exposures while some worked in non-diesel repair shops where there was little or no diesel exposure. Therefore, the exposure group was diluted by workers

without true diesel exposure, making it less likely to see an effect in this exposure group than if this dilution had not occurred. At the January 1996 Workshop (Workshop on Epidemiology-based Quantitative Risk Assessment/Analysis of the Garshick *et al.* 1988 Cohort Study), some attendees recommended exclusion of the shop workers in order to avoid misclassification due to very heterogeneous exposure. However, Dr. Crump (1996b) argues that, even so, shopworkers as a group probably had substantially higher exposures than other groups of railroad workers and also had greater opportunity for exposure to asbestos and welding fumes. Consequently he believes that the fact that shopworkers had lower lung cancer risks than other groups argues against a causal effect of diesel exposure in the cohort. Nevertheless, he has conducted analyses that exclude the shopworkers along with signalmen and hostlers, in addition to analyses that include the entire cohort.

B. Incomplete follow-up of the Garshick *et al.* cohort

It is agreed by Drs. Dawson and Crump that a substantial number of deaths occurring in the cohort during the last 4 years of follow-up were not identified in the original Garshick *et al.* (1988) study. Dr. Crump (1995, 1996a) asserted that because of the long latency of environmentally-induced lung cancer, data from 1977-1980 (the last four years of follow-up) are of critical importance in assessing potential effects of diesel exposure on lung cancer, and that no conclusions should be drawn from the Garshick *et al.* (1988) study until the follow-up is completed. He has also suggested that consideration be given to conducting a new study of the US railroad workers, correcting for the limitations of the Garshick *et al.* (1988) study, that could provide a much more definitive basis for evaluating any potential carcinogenic effects of diesel exposure. Dr. Dawson (1996b) agrees that much could be gained from additional data. Completion of the follow-up would reduce any risk of bias that might have occurred when using the full study period (1959-1980) with present data. In addition, Dr. Dawson has stated that a comprehensive new study, although costly and lengthy, could provide data to help reduce uncertainty about the relationship of diesel emissions to lung cancer. However, Dr. Dawson believes the available information provide a sufficient basis to support a more timely decision regarding protection of public health.

3. <u>Measure of Exposure</u>

Dr. Dawson used a single surrogate of diesel exhaust exposure in his analyses. Based on the data of Woskie *et al.* (1988a), Dr. Dawson used the concentration of total respirable particulate matter (RSP) adjusted by an estimate of the concentration attributable to environmental tobacco smoke. He refers to this as ETS-adjusted RSP. In contrast, Dr. Crump used 5 surrogates of diesel exposure in his analyses. Based on data from Woskie *et al.* (1988a) and Hammond *et al.* (1988), Dr. Crump used the following: RSP (concentration of total respirable particulate matter), ARP (RSP minus the concentration attributable to environmental tobacco smoke), AEM (an estimate of the organic fraction of RSP, minus the contribution of environmental tobacco smoke), TEX (AEM unadjusted for environmental tobacco smoke). These 4 surrogates were "climate-adjusted", based on the location of the railroad where a worker was last employed. Dr. Crump's fifth surrogate, UARP (ARP unadjusted for climate), is the same as Dr. Dawson's ETS-adjusted RSP.

4. Exposure Category Boundaries and Allocation of Cancer Deaths Across Exposure Groups

Dr. Crump divided exposure into 6 categories (with boundaries 0-485, 485-760, 760-1030, 1030-1300, 1300-1680, >1680 μg-years/m^3) for his primary results. He selected those boundaries for the exposure categories by equalizing lung cancer death rates in each of the 6 exposure categories using all 22 years (1959-1980) of the study. Dr. Dawson, however, divided cumulative exposure into 5 categories (with boundaries 0-450, 450-680, 680-950, 950-1520, >1520 μg-years/m^3). He selected the boundaries for the exposure categories by equalizing the number of cancer deaths in each of the 5 exposure categories utilizing lung cancer death rates for 1959-1977, omitting the last 4 years of the study.

Dr. Dawson equalized cancer deaths across exposure categories in order to have equal variances of the risk estimate for each exposure category. He stated (Dawson, 1996b) that one of the reasons his results differ from Dr. Crump's is because Dr. Crump excluded the last 4 years without establishing new exposure categories boundaries to re-equalize cancer deaths. This resulted in a decrease in the number of person-years with increasing exposure category. This decrease was sufficiently large that the number of cancer deaths also decreased with increasing exposure category even though the cancer incidence actually increased. Dr. Dawson suggested that the absence of statistically significant results by Dr. Crump is in part due to this manner of boundary selection. Dr. Crump disagreed. Dr. Crump (1996b) claims that the reason for the difference in results is not due to the fact that Crump's analysis did not equalize cancers across exposure groups, since he shows that analyses that equalize cancers but contain more groups give essentially the same non-significant trend as Crump's original analysis that did not equalize cancers. Dr. Crump argues that the reason for the difference in results is that in Dr. Dawson's categorization workers with the highest exposures (who did not experience an excess of lung cancer according to Figure 1 in Crump, 1996b) were grouped with workers having lower exposures, which diluted the effect of the high exposure data"

5. Trend of risk of lung cancer with increasing years of diesel exhaust exposure

Drs. Crump and Dawson continue to disagree about the interpretation of the trend of risk of lung cancer with increasing years of diesel exhaust exposure. According to Dr. Crump's 1991 reanalysis of the data using exposure assumptions close to that of Garshick *et al.* the relative risk of lung cancer decreased with increasing duration of exposure (shown in Figures 3 and 4 of Crump, 1996a). Dr. Crump stated (1996b) that the trend seen within the exposed population is not compatible with an effect of diesel exposure, despite the fact that the straight line fit to these data by Dr. Dawson has a statistically significant positive slope (Figure 4, Crump 1995 and 1996b). Dr. Crump does not believe that in this case the significant p-value demonstrates a biologically plausible effect of diesel exposure. Dr. Dawson thinks that the significant p-value may indicate an effect that is biologically plausible. He claimed (1996b) that this figure does not give an adequate visual representation of the results, exaggerating the role of the downward portion of the trend. According to Dr. Dawson, when the connection is made from the unexposed group to the lowest exposed group, it becomes clear that in none of the analyses does

the relative risk simply decrease with increasing exposure. Rather, relative risk is seen to increase from the unexposed group to the exposed groups, indicating an effect of exposure.

6. Interpretation of p-values in exploratory statistical analyses

Citing discussions in Statistical Analysis of Epidemiological Data, Second edition, 1996 by S. Selvin, Dr. Dawson (1996b) claimed that a p-value of less than 0.05 for a dose-response trend provided "rather definitive" evidence of an effect of diesel exposure. Dr. Crump (1996b) claimed, to the contrary, that this approach ignores the tendency for confounding to produce spurious results in a large and complex observational study. To illustrate, Dr. Crump applied the same battery of four statistical trend tests, which included the "interactive model", to lung cancer and four other causes of death considered unlikely to be related to diesel exposure. Eight of the sixteen p-values obtained from these latter four causes of death were smaller than 0.05, and the statistical evidence for a relationship with diesel exposure was stronger for three of these causes of death than it was for lung cancer. Dr. Crump believes that this exercise illustrates the potential for confounding to cause spurious statistical significance, and "highlights the need for a priori planning of analyses and reporting of all results, whether positive or negative, in order to present an unbiased picture." Dr. Crump claims that this is the approach he used in his collection of 80 analyses (Crump, 1996a), which involved different surrogates of diesel exposure, different methods of controlling for age and calendar year, and different subsets of the Garshick *et al.* cohort (including those that eliminated shopworkers). Consequently, Dr. Crump believes the fact that not one of these 80 analyses produced a statistically significant positive trend for lung cancer ($p < 0.05$) is highly notable.

* * *

The basis for the differences between the analyses and interpretations of the Garshick *et al.* (1988) cohort study by Drs. Crump and Dawson are summarized above. The issues are technically complex, and subject to interpretation. Furthermore, the way each of the specific issues is resolved has an impact on the risk assessment results.

A record of the correspondence between Drs. Crump and Dawson and related unpublished materials regarding analysis of the Garshick *et al.* (1988) cohort study is provided below.

1. Crump KS, Lambert T, Chen C. (1991). Assessment of Risk from Exposure to Diesel Engine Emissions. Prepared for U.S.EPA.

2. Garshick E. (1991): Letter to Dr. Chen, U.S.EPA, August 15, 1991.

3. Crump KS. (1994): Problems with using the Garshick *et al.* retrospective study of U.S. railroad workers for quantitative risk assessment. Presented at the workshop held by the California Environmental Protection Agency on September 12, 1994.

4. Dawson SV. (1995): Letter to U.S.EPA, April 27, 1995.

5. Crump KS. (1995): Letter to U.S.EPA, May 12, 1995.

6. Dawson SV. (1996a): Exposure-response analysis of the U.S. railroad worker cohort. Presented at the Diesel Exhaust Workshop, San Francisco, CA, January 29, 1996.

7. Crump KS. (1996a): Letter to Dr. Dawson, April 25, 1996.

8. Dawson SV. (1996b): Letter to Dr. Crump, June 13, 1996.

9. Crump KS. (1996b): Letter to Dr. Dawson, July 1, 1996.

10. Crump KS (1997): Letter to Dr. Dawson, January 6, 1997

APPENDIX F

Effect of Model Assumptions on Exposure - Risk Relationship for the Garshick *et al.* (1988) Cohort Study.

F.0 Introduction

The purpose of this appendix is to further evaluate the effect of model assumptions on the exposure-risk results. Use of the Garshick *et al*. (1988) cohort study to estimate unit risk is discussed in Section 7.3. Unit risk is obtained from the slope of the risk-exposure relationship at low values of exposure. Calculation of this slope is the primary objective of quantitative risk assessment when a threshold cannot be established. To provide a complete picture of the exposure-risk relationship, it is helpful to identify the character of any nonlinearity that exists. Standard epidemiological analyses provide methods for characterizing the trend of the relationship. The primary method is to conduct a linear regression and determine the quality of the fit. Another way is to introduce quadratic or higher order terms of exposure into the regression as was done in Appendix E. Yet another approach is to determine the trend for risk with categories of increasing exposure. Strictly visual observations or impressions may be influenced by an individual's expectation about the data or how the data have been plotted. In the discussions below, the influence of various assumptions and approaches on the calculation of the unit risk and characterization of the trend will be summarized.

The calculation of the unit risk estimate incorporates a number of assumptions. As indicated in Appendix E, there have been different findings and interpretations of the Garshick *et al*. cohort study. Efforts have been made to identify the differences in assumptions, approaches, and results. The factors discussed in Appendix F that appear to have most likely influenced the different results are: a) exposure pattern; b) control for age; and, c) adjusting the background concentration. Other factors that have been evaluated and either have less influence or have been ancillary to the reported disparate results are: d) exclusion of last 4 years of follow-up; e) exclusion of shopworkers; f) regression method g) model selection; and, h) detection lag. The influence of the factors and the preferred OEHHA analyses are summarized in Table F-1.

F.1 Exposure Pattern

As indicated in Section 7.3, the rate of dieselization of the U.S. railroads began to increase substantially in 1945. The number of railway workers exposed to diesel exhaust apparently increased during an initial period of increasing dieselization of U.S. locomotives from 1947 until 1959. Subsequently, diesel exhaust exposure apparently decreased following improved engine design, removal of excessively smoky diesel engines and improved engine efficiency. In light of this information, various investigators used different approaches in estimating exposure to railroad workers over time. As depicted in Figure F-1, there are three primary exposure patterns that have been used to describe the diesel exhaust exposure to railroad workers over time.

In the original Garshick *et al*. (1988) cohort study the authors chose 1959 as the effective start of diesel exhaust exposure in the cohort, because by that date 95% of the locomotives in the U.S.

were diesel-powered. However, the authors noted that some diesel exhaust exposure occurred earlier than 1959. They constructed a time-dependent model using years of diesel exposure as a surrogate for dose. This exposure pattern is depicted in Figure F-1A and this duration analysis is referred to as the "block" pattern.

In "An Assessment of Risk from Exposure to Diesel Engine Emission," Crump *et al*. (1991), estimated exposures prior to 1959. They assumed that exposures in each category increased linearly with time from 1945 through 1959. Their report stated that this linear increase parallels the overall rate at which the locomotive fleet became dieselized. This exposure pattern is depicted in Figure F-1B and is referred to as the ramp exposure pattern.

A further refinement of diesel exhaust exposure pattern is described in Section 7.3.2.2.2 to take into account the improvements in diesel engine design. The peak exposure during 1959 was assumed to decrease 3-fold to the 1983 exposure level; other assumptions regarding peak height have also been considered. Thus, the concentration was assumed to decline linearly from 1959 to 1980. This exposure pattern is depicted in Figure F-1C.

The choice of the pattern is an important basic consideration. In many instances the influence of other factors on the results is dependent on the exposure pattern. Consequently, when the influence of various factors is being evaluated, it is important to know the underlying exposure pattern being used. In the following discussion, the results of the factors will be presented in terms of the exposure pattern under consideration. It should also be noted that in general the cancer risks calculated for the block, ramp and roof patterns will decrease in that order. This is because the area under the concentration curve generally increases from block to ramp to roof patterns.

OEHHA prefers the use of a ramp or roof model to the block pattern. The major reason is to incorporate important available information on the rate of dieselization of locomotives. It is known that some workers were exposed to diesel exhaust prior to 1959. By incorporating a factor for dieselization in the model, this can be accounted for. Another reason for choosing the ramp and roof models is the susceptibility of the block pattern analysis to the method of controlling for age. This is discussed in greater detail below.

F.2 Control for Age

It is necessary to control for age in cancer epidemiology analyses because cancer rates are highly dependent upon age. The principles and concepts underlying the design analysis of cohort studies, including a discussion of the importance of controlling age is discussed in Breslow and Day (1987). The influence of method of control for age in these analyses is dependent on the exposure pattern under consideration. For this reason we discuss controlling for age separately for each exposure pattern.

F.2.1 Block-Pattern Duration Analysis

In computing risks for categories of exposure duration, the original Garshick *et al*. (1988) study controlled internally for age by using age-at-start of follow-up (i.e., 1959) along with the calendar-year time scale of a Cox regression. Their results exhibited a clear upward trend. This trend is reproduced in Figure F-2. OEHHA staff and Crump have been able to replicate these findings. From the data, OEHHA staff obtained a positive, significant dose-response slope (OEHHA 1994).

Crump *et al*. (1991) reported to the U.S.EPA a reanalysis of the individual cohort data. Crump *et al*. (1991) controlled internally for age by considering the age of the subjects in the cohort at the time of the observation (now referred to as "attained age"), and calendar year. These two categorical variables entered into the analysis using a multiplicative model. Crump *et al*. found "a statistically significant excess relative risk among exposed workers with two lowest categories of elapsed time but not in the two highest, and the excess did not increase monotonically with 'elapsed time,'" but no quantitative tests of trend appear in the report. This type of result is depicted in Figure F-3. OEHHA staff have been able to replicate these findings. Using a simple linear regression, OEHHA produced statistically significant positive slopes of risk against exposure time.

Garshick reported a further reanalysis of the cohort in a 1991 letter to U.S.EPA. He added a third variable, the age of the subject in the cohort at the time of the observation, to the previous control for age-at-start of study on the calendar year time scale of a Cox regression. He concluded that controlling for age in this manner in the analysis led to lower point estimates of the effects attributable to diesel exhaust with much less of a suggestion of an exposure-response relationship. Crump has commented that "when age is controlled for using a method that more accurately models the underlying age pattern, the trend relied upon by OEHHA is not present." However, no quantitative tests of trend appear in either report.

While the various methods to control for age may result in apparently different trends, it is important to acknowledge the large error bars around each point. When the results from the original and revised methods are overlaid on the graphical scale (see Figure F-4) it is clear that the two trends are not statistically distinguishable. This result supports the conclusion made in the 1991 letter by Garshick which states: "It can be seen that in the original estimates of the effects of diesel exposure and in the current estimates presented in Models 1 and 2, when the 95% confidence intervals are considered, there are no meaningful differences between estimates." However, OEHHA finds that it is best to exclude calculations from the block pattern in the range of risk because they do not consider pre-1959 exposures and the calculations are unduly sensitive to the method of controlling for age.

F.2.2 Ramp Exposure Pattern Analysis

In their report, Crump *et al*. (1991) used a ramp exposure in addition to the block pattern duration analysis. Using the ramp exposure pattern, Crump *et al*. (1991) controlled internally for age by using age at the time of observation ("attained age") along with calendar year in a Poisson

regression. They reported that "the dose response slope was negative for the complete cohort, engineers/firers, and conductors/brakemen. Discovery of an error in their use of a computer program (Dawson, 1995) resulted in reanalysis of the data. With correction of the error (Crump 1995, 1996, 1997) the calculated slopes of the linear relationship between risk and cumulative exposure while controlling for age and calendar year, were not statistically significant. OEHHA staff were able to confirm these results (Dawson, 1996). However, the analysis did not consider the unexposed workers to have zero exposures.

OEHHA also conducted a ramp exposure analysis where we controlled internally for age by using age at the time of observation ("attained age") along with calendar year, with a Poisson regression similar to the Crump (1995, 1996, 1997) analyses. However, our analysis assumed clerks to have zero exposure. In contrast to Crump's results, the slope obtained was statistically significant. In addition to controlling for age in the same manner as Crump *et al*. (1995, 1996, 1997), Appendix D also presents results for controlling for age by using age-at-start of follow-up (i.e., 1959) along with calendar-year. In this case where background was adjusted to zero, the slope was statistically significant. Appendix D also presented a variety of other ways of controlling for age and calendar year, including use of external controls. The slopes that resulted were not substantially different from those for the two other methods of control just cited. Consequently, while investigators may differ on visual interpretation of the plotted points, they provided a good fit to a linear dose-response trend when unexposed workers are adjusted to zero exposure.

F.2.3 Roof-Exposure Pattern Analysis

OEHHA conducted a roof exposure analysis, as described in Section 7 and Appendix D of this report. Section 7 used the roof exposure pattern with the published risk results of the Garshick *et al*. (1988) cohort study. The calculations in Section 7 converted the Garshick *et al*. (1988) results based on a block pattern duration of exposure to results based on a roof pattern describing cumulative exposure.

Appendix D used the roof exposure pattern with a number of different methods to control for age. One calculation included internal controls for age by using age at the time of observation ("attained age") along with calendar year in a Poisson regression, similar to that used for the ramp exposure pattern by Crump (1995, 1996, 1997). However, in contrast to the work of Crump our analysis subtracted background. The dose response slope was statistically significant (Table D-2). A second calculation controlled for age by using age at start of study and calendar year, as in Garshick *et al*. (1988). This analysis also produced statistically significant dose response slopes. Other calculations using different methods to control for age, produced similar results including the use of external controls (Table D-2).

F.2.4 Summary

The original analysis controlled for age by using age-at-start of follow-up along with a calendar-year time scale in a Cox regression (Garshick *et al*. 1988). OEHHA obtained a positive statistically significant dose-response slope with this data. In a follow-up analysis the third

variable, attained age, was added and the exposure-response trend was reportedly diminished (Garshick 1991), however, no quantitative tests of trend were reported. An external reanalysis using attained age and calendar-year reported a nonlinear trend in relative risks (Crump *et al.* 1991); however, no quantitative tests of trend were reported. The incorporation of various controls for age with the ramp exposure pattern did not have a substantial effect on the statistical significance of the slope, as long as background was adjusted to zero. Method of control for age did not effect the statistical significance of the slope for the roof exposure pattern. Consequently, only the block exposure pattern may be sensitive to the method of controlling for age.

F.3 Adjusting Background Concentration

Woskie *et al*. (1988a, 1988b) reported concentration as respirable particles (RSP) corrected for environmental tobacco smoke (ETS). The RSP was adjusted by Woskie *et al*. to remove the fraction of cigarette smoke; the resulting concentration is referred to as ARP. Clerks and signal maintainers were classified as unexposed to diesel exhaust by Garshick *et al*. (1988). A level of ARP exposure was reported in the Woskie *et al*. studies for the unexposed clerks/signalmen. As indicated by Woskie *et al*. (1988a) the clerks/signalmen ARP exposure was in the background range of the national average. Thus, the ARP exposure of the exposed groups reflected diesel exhaust exposure plus the ambient background experienced by the clerk/signalmen. Furthermore, as indicated by Hammond *et al*. (1988) the ARP exposures of the clerks "…almost certainly do not represent diesel exhaust from locomotives…" As pointed out by Garshick (1991) unless the clerks/signalmen group concentration is considered as nondiesel background, a cumulative exposure for the unexposed group will occur. This could result in a substantial number of unexposed individuals being intermixed with exposed individuals in the analysis. For example, assume a clerk's background exposure is misclassified as diesel exhaust exposure. Further, assume that a freight conductor's combined background plus diesel exhaust exposure is considered to be all diesel exhaust. This would make the presumed diesel exhaust exposure for 20 years of work as a clerk virtually identical to 10 years of exposure as a freight conductor. Thus, inclusion of the clerks/signalmen in the analysis in this manner could decrease the significance of the relative risk of the exposure groups.

The analyses in Garshick *et al*. (1988) compared lung cancer rates of unexposed railroad workers to the rates of exposed workers categorized by the duration of exposure. Garshick *et al*. did not actually use values of exposure concentration in their analysis, only the duration of exposure. In their study, the exposed workers had a continually increasing exposure while the unexposed workers were assumed to have zero exposure to diesel exhaust for the whole study period. Thus, for all the block pattern analyses, based on the original Garshick *et al*. results, the nondiesel background exposure is not an influencing factor since the unexposed workers have zero exposure.

As noted above, Crump *et al* (1991) and Crump (1995, 1996, 1997) performed analyses for the ramp exposure pattern based on concentration measurements made by Woskie *et al*. (1988a, 1988b) in the early 1980s. Those analyses used ARP without adjusting the background measurements of the unexposed workers to zero. Using this approach, Crump (1996) reported non-significant results for slope estimates, with attained age and calendar year.

Appendix D of the OEHHA report performed analyses for the ramp exposure pattern and adjusted the background measurements of the unexposed workers. Adjustment of the background exposure can occur in two ways. First, the background exposure of the clerks could be subtracted from the exposed workers. This assumes that both groups of workers were exposed to the same background concentration. Second, the background exposure of the clerks could be set to zero. This assumes that the clerks were exposed to background indoor air concentrations that were irrelevant to the exposed workers. These two adjustments would bracket the exposure concentration. Both types of calculations were conducted in the OEHHA analyses. Using attained age and calendar year, Appendix D reports significantly positive slope estimates. As a specific example, for internal controls on attained age and calendar year, the p-value fell from 0.037 to 0.006 when background was adjusted to zero exposure. For external controls the p-value fell from 0.068 to 0.010, thus becoming significant. Consequently, for dose-response slope results reported using the ramp exposure pattern, it is important to consider whether background was adjusted. In Appendix D of the OEHHA report analyses were also performed for the roof exposure pattern. In all of the reported roof pattern analyses, background was adjusted to zero. OEHHA's preferred approach is to adjust the background concentration of the clerks and signalmen to zero since the background particles are not from diesel exhaust. This would be consistent with the reported results of Woskie *et al.* (1988a, 1988b) and Hammond *et al.* (1988).

F.4 Exclusion of Last Four Years of Follow-up

In the original cohort study, Garshick *et al*. (1988) assessed the risk of lung cancer as a result of exposure to diesel exhaust for a cohort starting in 1959 and ending in 1980. In his reanalysis, Garshick (1991) also included the complete cohort in the assessment. Thus, in the reported Garshick *et al*. analyses the complete cohort was considered in the results. However, in recent comments on the OEHHA document, Dr. Garshick indicated that a major limitation for use of the study for risk assessment is the need to truncate the study in 1976 due to underascertainment of death in these years, thus eliminating important person-years of observation in workers with known exposure between 1977-1980.

In "An Assessment of Risk from Exposure to Diesel Engine Emission," Crump *et al*. (1991), the pattern of both lung cancer deaths and total deaths by calendar year, were studied. They reported that up through 1976, the age-specific death rates remained roughly constant; however, following 1976, the death rates begin to decrease precipitously with time. Death rates in the cohort begin to fall below U.S. rates in years subsequent to 1976. Thus, the cohort death rates decreased to values considerably below U.S. rates in subsequent years. The likely explanation for this drop-off in mortality is a lack of follow-up in the cohort. However, Crump *et al*. (1991) report that when the follow-up is stopped at the end of 1976, the results do not change materially. Thus, they concluded "the excess relative risk among the exposed workers found by Garshick *et al*. (1988) hold even if data after 1976 are omitted from the analysis." In subsequent analyses, results reported by Crump *et al*. (1991) do not suggest differences based on inclusion or exclusion of the last four years of follow-up.

OEHHA also conducted analyses including and excluding the last four years of follow-up. While the decision to include/exclude the last four years of follow-up had only a modest impact on the analysis, the most recent OEHHA calculations exclude the last four years of follow-up. OEHHA believes it is important to exclude the last four years of the cohort due to the lack of follow-up in the study. This will reduce the contribution of these individuals to the person-years at risk. Otherwise, the unaccounted for individuals are by default included in the category of individuals that remained alive and did not develop lung cancer.

F.5 Exclusion of Shopworkers

In the original Garshick *et al*. (1988) cohort study, the investigators reported results including and excluding the job classifications of shopworkers and hostlers. They reported that with both shopworkers and hostlers excluded from the analysis, the effect of diesel exhaust exposure remained significant and of comparable magnitude to the whole cohort. It is important to note that in the original analysis years of work in a diesel-exhaust-exposed job from 1959 to death or retirement served as a surrogate of cumulative exposure, and the worker exposures were not further subdivided by job category. As indicated by Garshick (1991), the problem with the published exposures is that the shopworkers who worked in the diesel repair shops shared job codes with workers in nondiesel shops where there was not diesel exhaust. Apparent exposure as a shopworker based on job code was then diluted with workers with the same job code, but without true exposure, making it less likely to see an effect in the shopworkers group. This point was emphasized by several investigators at the January 1996 Scientific Workshop.

In Crump *et al*. (1991), they utilized the exposure information published by Woskie *et al*. (1988a) for various railroad job categories. Based on this information, shopworkers were likely to have experienced the highest exposure to diesel exhaust. Crump conducted analyses including and excluding the shopworkers. When included, all shopworkers are assigned adjusted exposure concentrations based on work reported by Woskie *et al*. (1988a), without adjusting the background to zero. However, the presence of an effect does not seem to be affected by the inclusion or exclusion of shopworkers in the Crump *et al*. analyses.

In preparation of the 1994 OEHHA analyses, the Garshick (1991) comments on shopworkers addressed the concern that many of the designated shopworkers were not actually exposed to diesel exhaust; only half of them were considered exposed in the 1994 OEHHA analyses. After discussions in the 1996 Scientific Workshop, the shopworkers were excluded from the OEHHA analyses. In both analyses, the slopes were found to be generally statistically significant. Consequently, the incorporation of shopworkers in the OEHHA analyses did not appear to substantially affect the results. However, since the percent of shopworkers exposed to diesel exhaust is not known, it is preferred to exclude the shopworkers from the analysis.

F.6 Regression Method

Relative risks for the cohort study have been calculated primarily using two likelihood methods. The original Garshick *et al.* (1988) cohort study provided estimates of relative risk for death caused by lung cancer using partial likelihood methods as described by Cox. Exposure was

considered based upon diesel exhaust exposed job in 1959 and as a time-dependent covariate based on a cumulative number of years of diesel exhaust exposure from 1959 to 1980. The basic time scale in the Cox regression was calendar year. Crump (1997) reported that he was able to reproduce these results.

In the Crump *et al*. (1991) analysis of the block exposure pattern, estimates of relative risk for death caused by lung cancer were made using partial likelihood methods of Cox. However, the basic time scale was changed from calendar year to "attained age." Thus, the difference in these analyses is not the regression method per se, but the time scale used. They indicated that these results using this form of the Cox regression gave almost identical results to a corresponding analysis based on the Poisson regression. These results suggest that the choice of likelihood method, either those based on Cox or Poisson, do not substantially affect the outcome of the analyses. In the ramp exposure analysis, Crump *et al.* (1991) reported results using the Poisson regression.

OEHHA conducted the block exposure pattern, ramp exposure pattern and the roof exposure pattern analyses using the Poisson regression. This was done based on the reported results of Crump *et al*. (1991) and based on the comments by Breslow and Day (1987) that results from the two regression methods were remarkably similar.

Consequently, the method of regression did not appear to be an important factor in Garshick *et al* (1988) cohort study and the choice of regression method is relatively unimportant.

F.7 Model Selection

There are two broad classes of models that have been used in analyses of the Garshick *et al*. (1988) cohort study. They are : 1) general epidemiological models, and 2) a multistage model to incorporate the processes of carcinogenesis. General epidemiological models can combine the variables that explain incidence either multiplicatively or additively or some combination of the two. In multiplicative models, one calculates the relative risk, i.e. the risk relative to the background or control population. This has been the primary procedure used in the evaluation of the Garshick *et al*. (1988) cohort. The multistage model was suggested to address the influence of control for age. In the multistage model, cancer is assumed to occur only after a sequence of cell transformations has taken place. Thus, cancer incidence rates for older people are larger than those for younger people.

Garshick *et al*. (1988) and Garshick (1991) used general models in which incidence was assumed to be a multiplicative function of indicator variables for each duration of exposure category and for each category of age-at-start of follow-up and calendar year. Their results exhibited a clear upward trend. In 1991, Garshick also added an indicator variable for attained age. This led to lower point estimates of the effects attributed to diesel exhaust with much less of a suggestion of an exposure response relationship.

For both the block and ramp exposure patterns, Crump *et al*. (1991) and Crump (1995, 1996a,b) used general models in which incidence was assumed to be a multiplicative function of exposure

category and each category of attained age and calendar year. In analyses for the ramp pattern, exposure was evaluated in two ways; as a continuous variable or as categorical indicator variables. Using block exposure analysis, Crump *et al*. (1991) reported "a statistically significant excess relative risk among exposed workers with the two lowest categories of elapsed time but not in the two highest, and the excess did not increase monotonically with elapsed time." Using the ramp analysis, Crump reported the slopes were not statistically significant. However, the Crump *et al*. (1991) analysis did not adjust background exposures to zero. Crump *et al*. (1991) also reported the use of an additive model.

For the block exposure pattern, Section 7 reports the results for general multiplicative models with a variety of continuous and categorical forms of the variables. See Table 7-YY. The issues surrounding these analyses were described above. The multiplicative models produced statistically significant slopes. OEHHA staff have also tested an additive model and found that it produced statistically significant slopes.

In addition to the multiplicative model approach used by all investigators, OEHHA staff have also evaluated a biologically-based multistage model approach. This was done in response to suggestions made at the January 1996 Scientific Workshop to reduce the uncertainty in controlling for age. Appendix D reports the use of multistage cancer models of the type proposed by Armitage and Doll (1961). For both the ramp and the roof patterns of exposure, the best fit of the data to the multistage model was determined to be a model which used 7 sequential transitions necessary to produce the first cancer cell. The fit of the data with a model which used 6 sequential transitions was found to marginally fit the data. For the roof pattern, the fit of the risk slope further indicated that the stage that diesel exhaust acted on was the last stage of the cancer process or possibly the next to the last stage. For the ramp exposure pattern, the risk slopes were calculated for the assumption that diesel exhaust acted on the next to the last stage. These models generally produced highly significant slopes for both the ramp and roof patterns.

In analyses recently conducted by Crump (1997), he investigated the exposure-response relationship for the next-to-the-last-stage affected by diesel exhaust exposure. Crump (1997) reported the results did not indicate a progressive increase in lung cancer relative risk with increasing exposure. Despite these reported trends, the models generally obtained a statistically significant positive exposure-response slope. His results did not appear to be substantially affected by the choice of exposure pattern (ramp exposure or roof exposure), exclusion of shopworkers, or exclusion of last four years of follow-up.

The primary models used in most analyses have been general multiplicative models. Consequently, the model choice has not been a major factor producing differing results among investigators. There have been some analyses conducted with additive models and multistage models. As shown from the above results, the multistage models used by OEHHA provides more significant results. Thus, OEHHA finds that incorporation of both models in the analysis and the range of risk helps to characterize the range. In addition, the multistage model improves the control for age.

F.8 Detection Lag

As discussed in Section 6.2.4., most recognized human carcinogens have a "latent" period of at least 10 years after the initial exposure before their effects can be detected clinically. A concept related to latency is the mathematical concept of "lag". The term "lag" refers to time of first expression of disease until the time of death from disease. This lag period is important in cohort studies since the principle endpoint used in the calculations is death from the disease and not clinical detection of disease. This is the case in the Garshick *et al.* (1988) cohort study where lung cancer rates are based upon death certificates reporting lung cancer as the cause of death. Thus, the time of observation in the cohort study is the time of death. In the analyses conducted, the multistage model provides additional information to consider in the evaluation. In use of multistage models the lag refers to the development of the first cancer cell until the death of the individual. Thus, the definitions for the terms latency and lag are distinct and are not referring to precisely the same time periods.

For the Garshick *et al.* (1988) cohort study, when general models are used, detection lag is defined to be the time from the moment of carcinogenesis until death is recorded. Because the moment of carcinogenesis is not available directly, the detection lag was inferred from the modeling analysis. Garshick *et al.* (1988) made two different assumptions about the detection lag. One assumption was zero years, and the other assumption was five years. The assumption of zero years resulted in no evidence of a consistent exposure duration-response relationship. The assumption of five years for the detection lag led to significant results for the increase of risk with duration of exposure.

Crump *et al.* (1991) assumed a detection lag of either three or five years for the block exposure and the ramp exposure patterns. The results reported by Crump *et al.* (1991) do not suggest that the use of either a 3- or a 5-year lag period affected the results. In later analyses, Crump (1996a) used a 5-year lag period.

OEHHA has consistently used a 5-year lag period for the general models. Thus, in terms of the general models, the lag issue has been fairly consistently applied by Garshick *et al.* (1988), Crump *et al.* (1991, 1995, 1996a), and OEHHA (1997, 1998). Consequently, the issue of lag for the general models has not been a factor that has affected the findings or interpretations of the Garshick *et al.* (1988) cohort study.

As discussed above, OEHHA also evaluated the cohort study using a biologically-based multistage model. Due to the complexity of this model, the lag period had to be reconsidered. The ramp exposure pattern analyses used a five-year lag. For the roof pattern, the lag period depended on the specific analysis. The seven-stage model analysis with the next-to-the-last stage sensitive to diesel exhaust used a five year lag as the best fit lag value. The seven-stage model analysis with the last stage sensitive to diesel exhaust used 10 years for the detection lag. These choices were made to obtain the best fits of the model to the data. However, since the active stage differs in the two analyses, the two approaches appear to be fairly similar in their overall approach to the concepts of latency and lag. The use of the multistage models generally produced significant slopes for both the ramp and roof exposure pattern analyses. For the

multistage models with the last stage sensitive to diesel exhaust, the lag term affected the results. Using the 10-year lag increased the significance of the slope.

In analyses recently conducted by Crump (1997) he investigated the exposure-response relationship for the next-to-the-last-stage affected by diesel exhaust exposure. The results did not indicate a progressive increase in lung cancer relative risk with increasing exposure. Despite these trends, the models generally obtained a statistically significant slope. The lag period chosen, 5 or 10 years, did not appear to affect the results.

In the original Garshick *et al*. (1988) analysis, a five year lag was chosen due to its superior fit to the data over a zero year lag. Subsequently, most analyses have used five years for the lag term. OEHHA suggests that a five year lag be used unless a better fitting lag term is found in the analysis. Other lag terms may be identified as the analyses become more complex, as is the case for the multistage model analysis.

F.9 Issues of Nonlinearity

Trend is used in reference to the relative risk calculated for various categories of exposure. The slope is the best fit line through the points which can be tested for significance. Depending on the investigator and the analysis, the relationship between years of exposure and death from lung cancer may seem flat or decreases slightly. In many of these cases, especially in the analyses of OEHHA, the overall slope is still positive and statistically significant. It should be kept in mind that in analyses by OEHHA and Garshick, overall relative risk of the cohort is significantly increased.

The question arises as to whether a slope through all of the points is justified if the response seems to be flat or slightly decreasing at the high end exposures. OEHHA finds that calculating the slope is justified if the slope is found to be statistically significant. Furthermore, if the categorical trend doesn't provide a better fit than the use of a linear regression then the use of a slope is firmly justified. There should also be an attempt to reasonably explain any unusual points observed in the data set. Nonlinear portions of the dose-response curve have been identified in other OEHHA epidemiological analyses (i.e., nickel and arsenic).

Depending on the analysis, often the highest exposure point or points fell substantially below the dose-response line. There are at least four possible explanations for the change in the dose-response curve at higher exposures. They are described briefly here. First, there may be an attenuation of the pool of susceptibles (Cook, Doll & Fellingham, 1969). That is, those most sensitive to the risk of diesel exhaust may be leaving the cohort in higher proportions. Second, changes in the amount of exposure over time can impact the shape of the exposure-response curve (Cook, Doll and Fellingham, 1969). The decline in exposure concentration over time could contribute to decreases in cancer risk. Third, the actual model may be additive rather than multiplicative, or may have an additive component. This was suggested by Dr. Thun at the Society for Risk Analysis Symposium (Monterey, 1997). Our limited use of an additive model suggests an improved fit to the data. Fourth, this may be a reflection of within-group variation over a small dose range. Generally, dose-response is thought of in terms of toxicology where

there is a log scale to accommodate a dose-response over a larger range of doses. In this case, the dose groups are spaced over a narrow range. The deviations from linearity may simply reflect interindividual variability. All of the above factors could contribute to the non-linearities in the dose-response curve. Thus, while the specific factors cannot be identified, it is important to note that there are reasonable explanations for the apparently complex results. Finally, statistical analysis indicates whether the departure substantially affects the use of a slope.

F.10 Summary

Clearly, the most important factor influencing the use of the Garshick *et al.* (1988) cohort study for risk assessment is the uncertainty in exposure assessment. As indicated above, while the overall results of the study remain unchanged, the exposure pattern assumed (along with other factors) can greatly modify the dose-response results. Dr. Garshick in his comments on the document, indicated that in the assignment of past exposure there is great uncertainty regarding the level of exposure, and which workers were actually exposed to diesel exhaust before 1959. However, it is known that workers were exposed to diesel exhaust prior to 1959. For this reason, OEHHA finds that the best exposure scenarios incorporate the rate of dieselization to estimate pre-1959 exposures. The document presents a range of exposure scenarios using the ramp and roof patterns to capture the uncertainty in the exposure ascertainment. Other factors thought to most influence the differing results of the Garshick cohort analysis were the methods of control for age and the adjustment of background exposures for unexposed workers. The influence of these two factors is dependent upon the exposure pattern being considered. Control for age has the largest effect for the block pattern analysis. Adjustment of background concentration is an important factor in interpreting results from the ramp pattern analyses. Two other issues that were considered to be potentially important factors were exclusion of the last four years of the cohort due to lack of follow-up and exclusion of the shopworkers. In our evaluation, while they only play a relatively minor role in the results, these subgroups should be excluded from the analyses. This is consistent with the need to address important reported limitations of the cohort study. A fifth factor, the regression method employed was thought to be relatively unimportant. Based on current information available, that appears to be the case.

As a result of public comments and the 1996 Scientific Workshop, it was suggested that the control for age issue could be minimized by using a biologically-based analysis. For this reason, OEHHA conducted limited additional analyses using an Armitage-Doll approach. Incorporation of this approach tended to further improve the significance of the results. However, the approach also required additional assumptions to be incorporated, for example, adjustment of the lag variable. Thus, while the incorporation of this approach tended to support OEHHA's previous analyses, it is a fairly new area of analysis and has not been a primary reason for reported differences among investigators. For this reason, OEHHA finds it useful to report both general and multistage model results to describe the range of risks.

The preferred OEHHA approaches tend to minimize the nonlinearity and produce significant cancer risk slopes. The OEHHA assumptions have focused on incorporating available data to improve model assumptions (e.g., rate of dieselization, subtraction of background exposures) evaluating the sensitivity of alternate approaches (e.g., ramp and roof patterns, general and

multistage models), and addressing limitations in the available data (e.g., shopworker misclassification, poor follow-up in the last four years). Consequently, even with the limitations and uncertainties present in the cohort study, we find that it contributes useful and important scientific information to help characterize the range of cancer risks posed by diesel exhaust exposure by including those approaches that minimize the nonlinearity.

A record of the correspondence between Drs. Crump and Dawson and related unpublished materials regarding analysis of the Garshick *et al.* (1988) cohort study is provided below.

1. Crump KS, Lambert T, Chen C. (1991). Assessment of Risk from Exposure to Diesel Engine Emissions. Prepared for U.S.EPA.

2. Garshick E. (1991): Letter to Dr. Chen, U.S.EPA, August 15, 1991.

3. Crump KS. (1994): Problems with using the Garshick *et al*. retrospective study of U.S. railroad workers for quantitative risk assessment. Presented at the workshop held by the California Environmental Protection Agency on September 12, 1994.

4. Dawson SV. (1995): Letter to U.S.EPA, April 27, 1995.

5. Crump KS. (1995): Letter to U.S.EPA, May 12, 1995.

6. Dawson SV. (1996a): Exposure-response analysis of the U.S. railroad worker cohort. Presented at the Diesel Exhaust Workshop, San Francisco, CA, January 29, 1996.

7. Crump KS. (1996a): Letter to Dr. Dawson, April 25, 1996.

8. Dawson SV. (1996b): Letter to Dr. Crump, June 13, 1996.

9. Crump KS. (1996b): Letter to Dr. Dawson, July 1, 1996.

10 Crump KS (1997): Letter to Dr. Dawson, January 6, 1997

Table F-1. Summary of Effects of Assumptions Used in Determining Exposure-Risk Relationships for Garshick *et al.* (1988) Cohort Study

Factor	Influence on Results	Importance for Differing Conclusions	OEHHA's Preferred Approach
Exposure pattern	Patterns are described in Figure F-1. They greatly influence the results in combination with the method of controlling for age. Garshick et al. (1988) described a block exposure pattern. Ramped approaches have been added to incorporate increases in railroad dieselization. A roof pattern approach was used to incorporate dieselization until 1959 and reduced engine emissions following 1959. Roof results were more likely to report a positive dose response (1997). The roof pattern generally assumes greater exposure, and therefore, less risk.	High	Use ramp and roof patterns as best exposure estimates
Control for age	Method of control appears to produce substantially different results for the block exposure pattern. Original control with age-at-start of follow-up along with the calendar-year time scale of a Cox regression, exhibited a clear upward trend (Figure F-2). Adding attained age resulted in much less of a suggestion of an exposure-response relationship (Figure F-3). However, the revised estimates are not statistically different from the original estimates (Figure F-4). Various controls for age have only a modest effect for the roof and ramp exposure patterns using general multiplicative models.	High for block pattern. Low for ramp or roof pattern.	Either method for roof or ramp pattern

Table F-1. Summary of Effects of Assumptions Used in Determining Exposure-Risk Relationships for Garshick *et al.* (1988) Cohort Study (continued).

Factor	Influence on Results	Importance for Differing Conclusions	OEHHA's Preferred Approach
Exclusion of last 4 years of follow-up	There is a substantial drop off in mortality in the cohort due to lack of follow-up for the last four years. This has only minor influence on analysis; likely reducing significance of the results. Original study included last four years. Subsequent analyses have been conducted with and without last four years with little impact on the results.	Low	Exclude last 4 years of follow-up
Exclusion of shopworkers	It is not known what proportion of shopworkers were exposed to diesel exhaust. Heterogeneity of the exposure group brings into question the nature of their exposure. However, the inclusion or exclusion of shopworkers in the analysis does not seem to determine the presence or absence of a significant result.	Low	Exclude shopworkers
Regression method	Has negligible influence on the results. The relative risks for the cohort have been calculated primarily using two likelihood methods, those of Cox and Poisson. The results from the two methods are considered equivalent.	None	Either
Model Selection	Most analyses were based on multiplicative models. Other factors used in the model such as control for age, and subtraction of background influenced results. OEHHA has conducted additional analyses using biologically-based, multistage models. The results provide significant slopes but suggest slightly lower risks to the public.	Low	Both
Detection Lag	Five year lag generally used in all cases for the multiplicative models. In OEHHA's additional biologically-based analyses a 10-year lag was used for last stage cancer model. Analysis by Crump (1997) suggests lag period does not substantially affect results.	Low	Use best fit

Table F-2. Primary Assumptions Used in Various Analyses of the Garshick *et al*. (1988) Cohort Study.

Factor	Garshick *et al.* (1988)	Garshick (1991)	Crump *et al.* (1991) Crump (1995, 1996)	Crump(1997)	OEHHA (1994)	OEHHA (1997)	OEHHA (1998)
Exposure pattern	Block	Block	Block, Ramp	Block, Ramp, Roof	Block	Ramp, Roof	Ramp, Roof
Control for age	Age-at-start, calendar year	Age-at-start, calendar year, attained age	Age-at-start, calendar year	Age-at-start, calendar year	Age-at-start, calendar year	Age-at-start, calendar year, attained age	Age-at-start, calendar year, attained age
Adjust background concentration to zero	Yes	Yes	Block-yes Ramp-no	Block-yes Ramp-no Roof-yes	Yes	Yes	Yes
Exclusion of last 4 years of follow-up	No	No	Both ways	Both ways	No	Both ways	Yes
Exclusion of shopworkers	Both ways	Both ways	Both ways	Both ways	No, but corrected for it	Yes	Yes
Regression method	Cox	Cox	Poisson, Cox	Poisson, Cox	Poisson	Poisson	Poisson
Model selection	Multiplicative	Multiplicative	Multiplicative, additive	Multiplicative, multistage	Multiplicative	Multiplicative, multistage	Multiplicative, multistage
Detection lag	0, 5 years	5 years	3, 5 years	5, 10 years	5 years	5, 10 years	5, 10 years

Figure F-1: Different Exposure Patterns

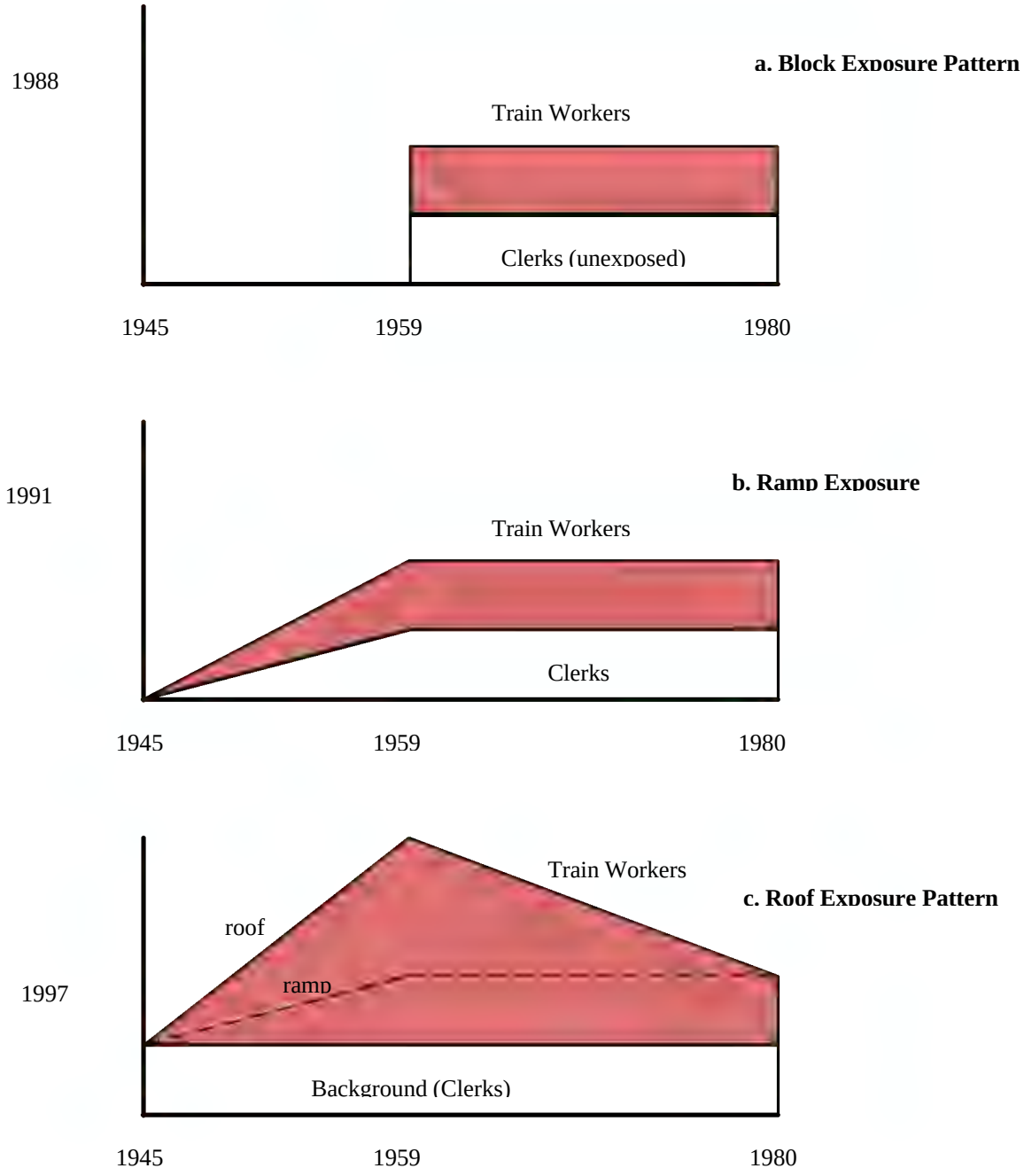
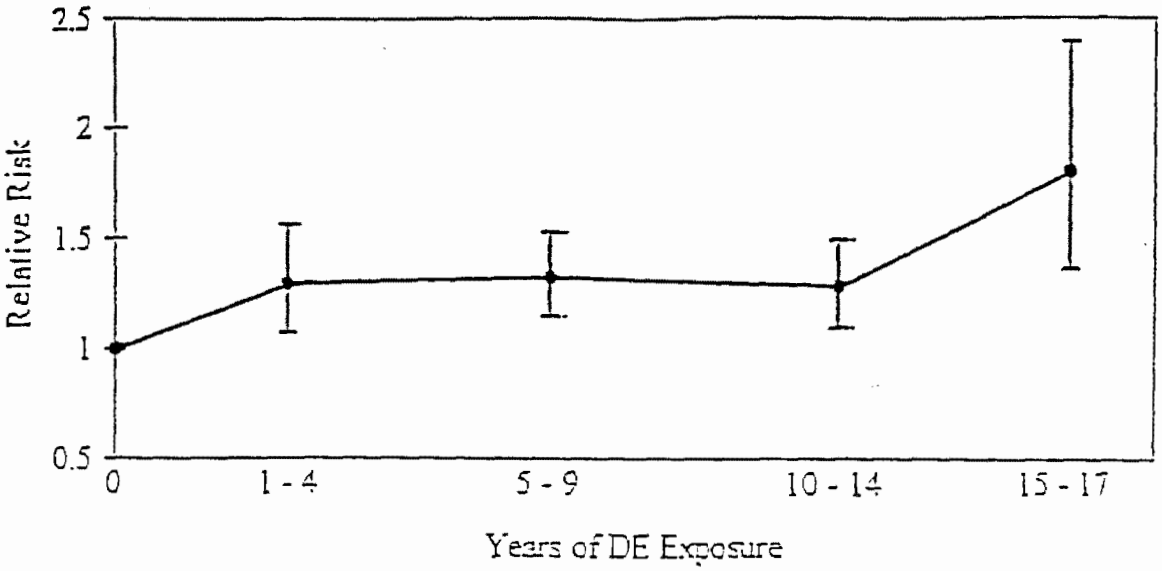
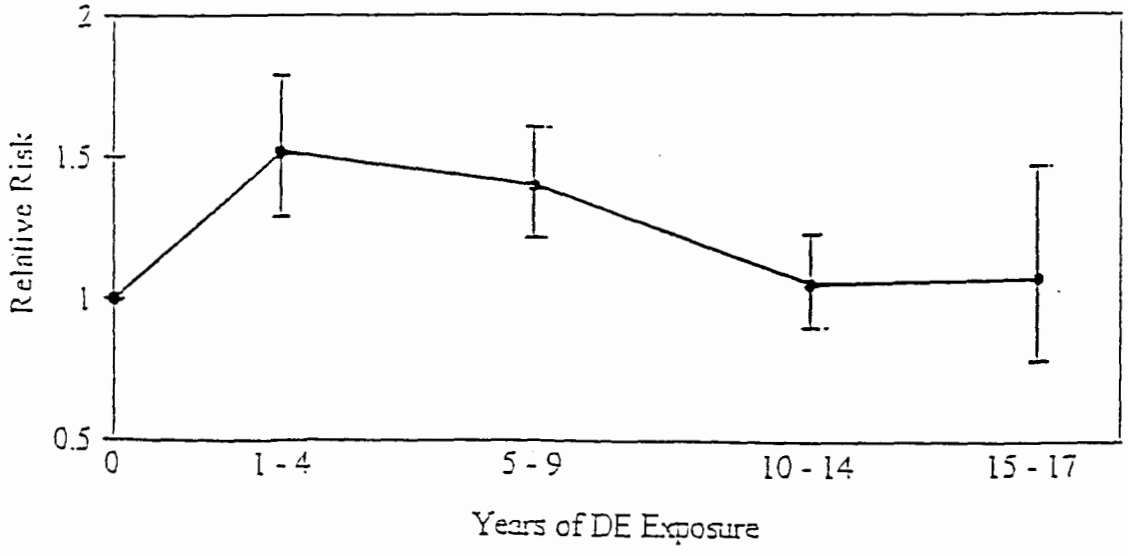


Figure F-2. Trend in Lung Cancer Relative Risk with Duration of Exposure, Controlling for Age



Cox regression using calendar year as a time variable, using 5 category variables for age in 1959, and counting any exposure in a year as a full year of exposure.

Figure F-3. Trend in Lung Cancer Relative Risk with Duration of Exposure, Controlling for Attained Age



Cox regression using calendar year as a time variable, using 5 category variables for attained age, and accounting for exposure as number of months worked. Excluding shopworkers and hostlers.

Figure F-4: Comparison of Trends of Relative Risk Using the Different Covariates For Age and Different Methods of Calculating Duration Block Exposure Pattern. Excluding Shopworkers and Hostlers

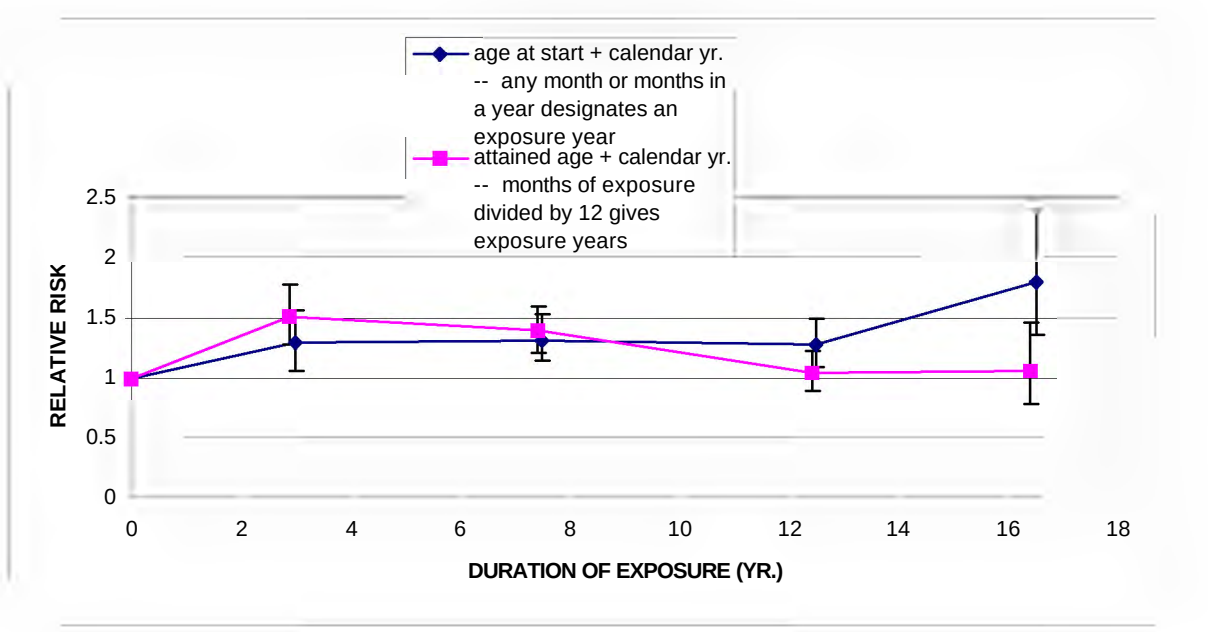
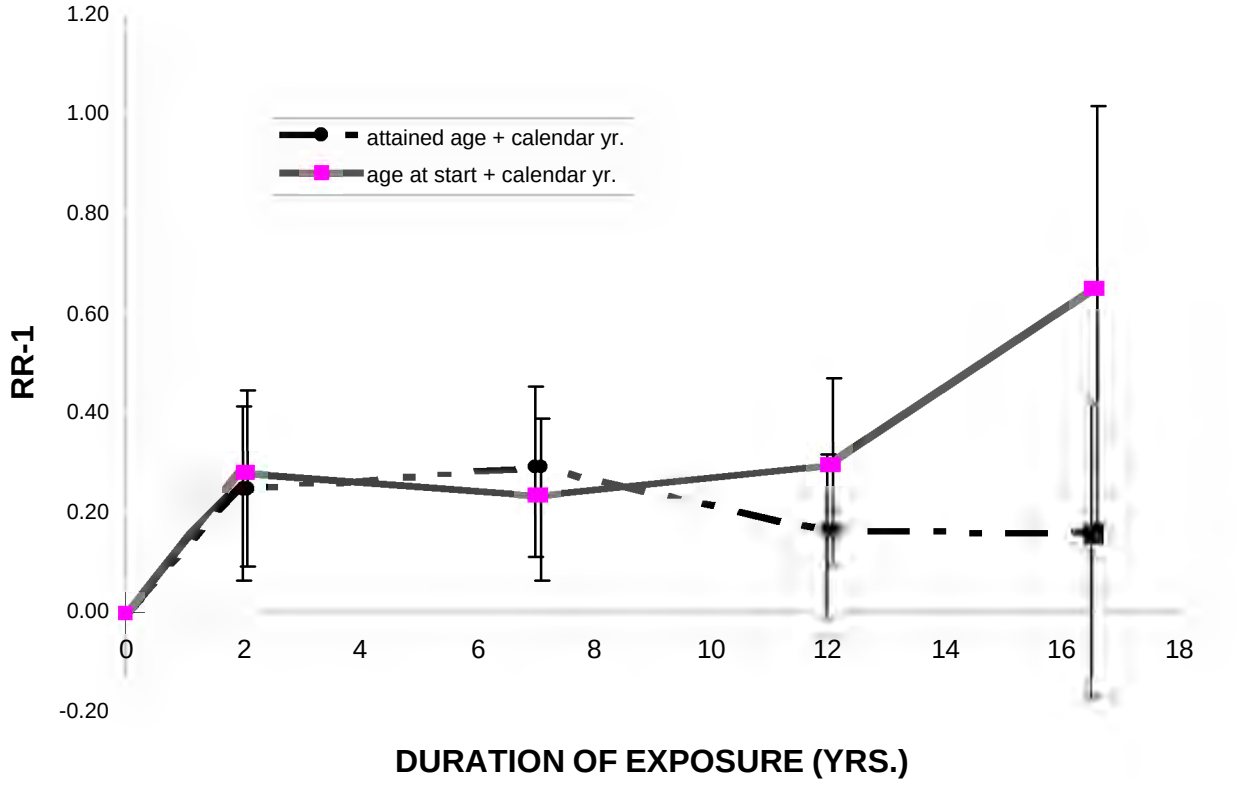


Figure F-5: Trend of Excess Relative Risk With Exposure Stratified by Five Calendar Year Categories and Either Five 5-Year Categories of Starting Age or Five 5-Year Categories of Attained Age



APPENDIX G

QUANTITATIVE CANCER RISK ASSESSMENTS BASED ON RAT LUNG TUMOR DATA AND COMPARATIVE POTENCY ANALYSES

This appendix provides estimates of the risk of humans developing cancer due to the inhalation of diesel exhaust in the atmosphere based on animal studies and on comparative potency analyses. It is useful to discuss the estimates of unit risk and the uncertainties in the estimates from the rat lung tumor data and compare them to that estimated from the epidemiological data. Presentation of the unit risk estimates based on the animal data is followed by a discussion of the uncertainties in using the animal data. There are a number of uncertainties regarding extrapolation of animal carcinogenicity data (see Section G.2.8). Among the uncertainties are possible differences in pharmacokinetics and pharmacodynamics. The mechanism(s) of action of diesel particle-induced rat lung tumorigenesis are not fully characterized. Soot particle overload may play a role in the induction of lung tumors in the rat studies. However, the role of the genotoxic constituents of diesel exhaust in the development of rat lung tumors is as yet undefined. Some scientists argue that the diesel exhaust-induced rat lung tumor data are not applicable to humans. In general, the rat has been a reasonably good predictor of human lung carcinogens and better than the mouse or hamster. There are still data gaps in the understanding of how diesel exhaust induces lung tumors in the rat.

G.1 HUMAN RISK ESTIMATES USING A RODENT BIOASSAY

Diesel exhaust particulate matter and its extracts have been shown to be carcinogenic in rats at exposures in the mg/m^3 range. With a sufficient number of animals, inhalation studies utilizing exposures greater than or equal to 2.2 mg/m^3 and observation periods of approximately 2 years have consistently demonstrated significant increases in pulmonary tumors in rats. Tumorigenicity results in mice have been mixed. To date, the results in Syrian hamsters have been negative. The hamster model is generally resistant to the development of lung tumors from known human carcinogens (Heinrich *et al*., 1986a; IARC, 1996). Table 6-1 summarizes the experimental studies of diesel exhaust carcinogenicity following inhalation exposure.

After specifying the measure of exposure to be used for the animal studies and selecting the most relevant animal study, this section interpolates quantitative estimates of the carcinogenic risk of diesel exhaust at the low exposures that humans are likely to experience. These animal results are then extrapolated to humans using standard scaling methods.

G.2 SPECIFICATION OF THE MEASURE OF EXPOSURE

Diesel exhaust carcinogenicity bioassays using rats have consistently indicated that exposure to unfiltered diesel exhaust at a sufficient concentration for a sufficient length of time results in the induction of lung tumors. In contrast, studies examining the tumorigenicity of filtered diesel exhaust have tended to provide negative results; the exceptions are studies by Heinrich *et al*. (1986a) conducted in female NMRI mice, and by Iwai *et al*. (1986) reporting that filtered and unfiltered diesel exhaust increased the incidence of splenic malignant lymphomas in female

Fischer 344 rats. Therefore, regardless of specific engine source, this quantitative risk assessment uses diesel exhaust particulate matter as the dose metric of diesel exhaust exposure because of its consistent association with diesel exhaust-induced tumorigenicity.

Two related measures of cumulative exposure to diesel exhaust will be used in developing a quantitative relationship between the risk of cancer and the exposure of the test animals to diesel exhaust. Cumulative exposure considers concentration or lung burden together with duration of exposure. The first measure is based on the cumulative mass of particulate matter (soot) deposited in the lung due to inhaling diesel exhaust, although it is usually expressed as a concentration over time. The second measure is the cumulative exposure to the lung burden due to inhalation of diesel exhaust. The lung burden is the mass of particulate matter that is in or on the lung tissue at any particular time.

G.2.1 DETERMINATION OF CUMULATIVE MASS DEPOSITED IN THE LUNG

The basis for the first exposure measure is the cumulative mass deposited in the lung. That mass is inferred from standard measurements external to the lung, as follows:

(1) Atmospheric concentration x rate of inhalation of air volume = rate of intake of particle mass.
(2) The time period over which concentration is constant x that concentration = the mass of particles inhaled over that time period.
(3) The sum of those masses for successive time periods = cumulative mass inhaled.
(4) The cumulative mass inhaled x the effective deposition efficiency of the lung = the mass deposited in the lung.

Using this basis, mass deposited in the lung, is conceptually very helpful in clarifying the scaling procedure in extrapolating results from rodents to humans, below. It is convenient in practice, as in this report, to use instead the essentially equivalent measure, cumulative exposure, which is proportional to the mass deposited. The proportionality constant is the volumetric inhalation rate times the lung deposition efficiency. The cumulative exposure is then determined by summing or integrating directly from the time course of concentration and has units of time x concentration ($yr\text{-}\mu g/m^3$). The cumulative exposure is essentially the standard assumption of California cancer guidelines (DHS, 1985).

G.2.3 DETERMINATION OF CUMULATIVE EXPOSURE TO LUNG BURDEN

For the time-to-tumor analysis of the rat data of Mauderly *et al*. (1987a) below, the lung burden in rats is inferred from the in situ measurements of Henderson *et al*. (1987). Their method measured light extinction in lung homogenates due to the deposited diesel exhaust particles. The calibration procedure measured light extinction for unexposed lung homogenates spiked with known masses of diesel exhaust particulate. Their data provides a time course of lung burden at six-month intervals.

In this report the time course of lung burden from these data is used only to obtain the average value of lung burden over the first two years of the rats' lifetime. The available computer

programs used below to calculate the relationship of risk to exposure assume a constant concentration over the experimental lifetime. Thus the average value of lung burden was used as the constant concentration for those programs.

For the calculations using human exposures, lung burden was considered sufficiently small so as not to require use of a special model. Small lung burdens may be assumed to be linear in atmospheric concentration, as in the model of Figure G-1. Then atmospheric concentration may be used directly or as a surrogate for lung burden.

G.2.4 SELECTION OF ANIMAL STUDY FOR QUANTITATIVE RISK ASSESSMENT

The most appropriate animal inhalation study for calculating risks was selected from a survey of studies. Of the three studies showing positive lung tumor responses in mice (Pepelko and Peirano, 1983; Heinrich *et al*., 1986a; Takemoto *et al*., 1986), only the study of Takemoto *et al*. (1986) can be considered even marginally useful for risk calculations. The study of Pepelko and Peirano (1983) extended only to partial lifetimes and did not provide a straight-forward dose-response relationship. The study of Heinrich *et al*. (1986a) did not attain adequate survival in the unfiltered diesel exhaust exposure group. Takemoto *et al*. (1986) exposed ICR and C57BL/6N mice to 0 or 2-4 mg/m^3 diesel exhaust 4 h/d, 4 d/wk from birth to 24-28 months of age. Animals were serially sacrificed at 3, 6, 12, 18 and 24-28 months. The study design was limited by having only one diesel exhaust exposure group, and that exposure was not well regulated.

Seven studies reported positive lung tumor response in rats (Heinrich *et al*., 1986a; Mauderly *et al*., 1987a; Iwai *et al*., 1986; Ishinishi *et al*., 1986a; Brightwell *et al*., 1986, 1989; Heinrich *et al*., 1995; Nikula *et al*., 1995). Only one of these studies (Heinrich *et al*., 1986a) did not use serial sacrifice. This study was also the only one that used Wistar rats and that reported a preponderance of benign over malignant tumors. The steepening dose-response relationship observed in the five studies with more than one exposure group (Mauderly *et al*., 1987a; Ishinishi *et al*., 1986a; Brightwell *et al*., 1986, 1989; Heinrich *et al*., 1995; Nikula *et al*., 1995) indicates that use of the two studies with only one exposure group (Heinrich *et al*., 1986a and Iwai *et al*., 1986) would not permit accurate modeling of the implied non-linear response. Of the five studies with several exposure groups, complete time-to-death data were available for only one (Mauderly *et al*., 1987a). See Table G-1 for a summary of data generated from that study.

The time-to-tumor analysis provides the most accurate characterization for the multistage model and is expected to produce the best model fit if appropriate data are available. Thus, time-to-tumor analyses of multistage models of carcinogenesis were chosen to predict risk from rat data at low exposures, using the available data of Mauderly *et al*. (1987a) on all lung tumors in rats. That study had the advantages of available time-to-tumor data, a substantial number of animals, an extended study period and relevant auxiliary data.

While the data of Mauderly *et al*. (1987a) were determined to be most applicable among rat data sets to estimate cancer potency, a comparative evaluation of several multiple dose experiments with rats was also conducted.

G.2.4.1 COMPARATIVE ANALYSIS OF FIVE RAT BIOASSAYS

Five studies in rats with multiple diesel exhaust exposure groups were evaluated (Table G-2 and G-3). Squamous cysts were excluded from the analysis of data of Mauderly *et al*. (1987a), except for the linearized multistage analysis based on ambient concentrations in which two runs were conducted one with and one without squamous cell cysts. Lung tumor potency was estimated from these studies using a single statistical model. Ninety five percent upper confidence limit estimates were compared to assess the consistency of the available studies in predicting an upper-bound on the incidence of carcinogenic effects.

The model used was the linearized multistage (LMS) model that has been extensively used by OEHHA, U.S. EPA, and other public health organizations (DHS, 1985, U.S. EPA, 1987, WHO, 1996).

Unit cancer risk estimates were derived from the five studies using two different measures of dose (Table G-3). First, the relationship between ambient concentration and lung tumor formation was examined. Second, lung tumors were analyzed as a function of cumulative lung burden over time. The latter dose measure was assumed on theoretical grounds to be a better predictor of tumorigenicity. Lung burden estimates for Brightwell *et al*. (1989), Heinrich *et al*. (1995), Ishinishi *et al*. (1986a), and Mauderly *et al*. (1987a) were derived from the model of Yu and associates (1991) and are equivalent to those presented by U.S. EPA (1998) and WHO (1996). Average lung burden for the study of Nikula and associates (1995) was estimated by time-weighted averaging of data and assuming a rat alveolar surface area of 3400 cm^2 (U.S. EPA, 1994). Extrapolation from rat risk per unit lung burden to corresponding human risk per unit concentration human exposure was based on the factor 510 cm^2 alveolar surface area per cubic meter inhaled (U.S. EPA, 1996).

The results of this evaluation are presented in Table G-3. The three studies associated with the higher potency estimates had high-dose exposures of 6.5 to 7.1 mg/m^3, whereas, the two studies with lower estimates had high-dose exposures of 3.7 mg/m^3, though the significance of this observation is unknown.

Sampling error alone may account for the interstudy differences. The Brightwell results appear to differ somewhat from the other studies. Excluding the Brightwell study, the estimates from the four remaining studies varied over only a two-fold range. Significant differences have been observed between F344 rats obtained from different sources, which could have had some influence on the results. For example, a Japanese F344 substrain is dipeptidyl peptidase IV deficient whereas other strains have normal activity (Watanabe *et al*., 1993).

All studies used F344 rats, except for that of Heinrich, for which Wistar rats were studied. The Wistar rat study resulted in cancer potency estimates in the middle of the range of values derived from studies of F344 rats.

For the Heinrich and Brightwell studies, the lung burden data provided a substantially better fit to the LMS model than air concentration data. For the Mauderly study, the air concentration fit

the model slightly better than the lung burden data. The Ishinishi data fit the LMS model equally well using either dosimetric assumption. For all 5 studies both dose measures provided an adequate fit to the LMS model.

The 10-fold range of UCLs obtained for the comparative study, 16 to 160 cases per million persons exposed to 1 $\mu g/m^3$ diesel particulate matter over a lifetime, overlaps in its upper end the 5-fold range obtained for the analysis of the Mauderly *et al*. (1987a) study, 54 to 280. When narrowed to consideration of the results for lung burden only, the 5-fold range for the comparative study, 16 to 77 is very close to the range for Mauderly *et al*. (1987a), 15-54.

These estimates do not address potential differences between rat and human susceptibility to diesel-caused lung tumor formation. The animal data may also project misleadingly low estimates of upper-bound human unit cancer risks as the human population may be considerably more variable in response than that observed in an in-bred laboratory animal population.

The rat studies however demonstrate that multiple studies by different researchers in one species to known diesel exhaust particulate for different concentrations and temporal patterns lead to a relatively consistent projection of unit cancer risks.

G.2.5 INTERPOLATION OF RAT BIOASSAY RISKS AT LOW EXPOSURES

The analysis uses two forms of the multistage model to fit the time-to-tumor data kindly provided by Dr. Mauderly. The first form was the Weibull (in time) multistage model, and the second was the simplified Moolgavkar model, which takes cell proliferation into account. The analyses were run using the TOX_RISK program (ICF Kaiser, 1993). Each form requires a constant exposure level or dose as input. Both forms start with the same basic expression for risk of cancer at time t (Lawless, 1982):

$$P = 1 - \exp(-H_c(D,t)), \qquad (1)$$

where

t = time since beginning exposure (wks),
P = probability of carcinogenesis,
$\exp(x) = e^x$ = exponential function of x,
$H_c(D,t)$ = cumulative hazard function for the particular form, c, of the model,
D = dose (lifetime-$\mu g/m^3$ or lifetime-mg).

Weibull multistage model. For this form of the model the hazard function assumes a dose polynomial multiplied by a power of the exposure time. (ICF Kaiser, 1993).

$$H_w(D,t) = (r_0 + r_1 D + r_2 D^2 + \ldots + r_n D^n)[h(t-s_1)]^k, \qquad (2)$$

where

r_i = the i-th (constant, non-negative) coefficient in the dose polynomial,

n = the order of the dose polynomial,
s_1 = time from formation of a tumor cell until the resulting tumor is detectable,
k = a power, not necessarily computed as an integer.
$h(x) = \begin{cases} 0, & x<0, \\ x, & x>0. \end{cases}$

Simplified Moolgavkar Model. For low probability of cancer, the approximate expression for this form of the model assumes that the hazard function is a second degree polynomial in dose multiplied by an exponential time function representing the effect of cell proliferation upon the risk of mutation (Thorslund *et al*. 1987; ICF Kaiser, 1993).

$$H_m(D,t) =$$

$$\{v_0 + v_1 h(D-D_1) + v_2[h(D-D_1)]^2\} \{\exp[Gh(t-s_1)]-Gh(t-s_1)-1\}/G^2, \qquad (3)$$

where
v_i = constants (i = 0,1,2,3,4,5)
$G = v_3 + v_4[h(D-D_0)]^v 5$ = cell proliferation factor.
D_0, D_1 = dose thresholds, the values of dose below which the indicated term does not contribute to the risk.

Estimation of parameters. The analysis estimates the parameters of the above models by forming the log-likelihood function in each case and then obtaining maximum likelihood estimates (MLEs) for the best fit of the model to the data. The form of the likelihood function for this survival analysis takes account of time of death with lung tumor as well as censoring of such observations due to any deaths in which lung tumors were not detected. Likelihood methods are further used to obtain the 95% upper confidence limit (UCL) of unit risk, for the slope of the probability expression as a function of exposure at low values of exposure (ICF Kaiser, 1993). This value is used to characterize predictions of unit risk.

Squamous cysts were excluded from the analysis. Their biological significance for the purposes of estimating human cancer potency is controversial. Inclusion of the squamous cysts in the risk assessment would result in approximately 30% higher potency.
The MLE values of model parameters and the UCLs for unit risk are presented in Table 7-4. The analyses fitted both forms of the model with both measures of dose input: the concentration of the inhaled particles and the average lung burden of particles. All four of these computations gave the value zero for latency period. This is the detection lag, the time from carcinogenesis to detection of the tumor. Also, the computations for the Moolgavkar model gave the value zero for both values of dose threshold (D_0 & D_1) with both measures of dose input.

The Weibull results were checked using the WEIBULL85 program, which led to corrections of the TOX_RISK program and to more assured interpretations of the effect of the complex system

of conversions and times in TOX_RISK. The conversions were also checked in both the Weibull and Moolgavkar routines by applying the desired scaling directly to the raw MLE parameter, q_1, obtained from the fitting process, to compare to the computer scaled MLE value ultimately reported by TOX_RISK for the target conditions.

G.2.6 METHODS OF EXTRAPOLATING RISKS FROM RODENTS TO HUMANS

The extrapolation from rodents to humans depends upon selection of a time scale and a dose metric that are assumed to give identical risk estimates for rats and humans. Extrapolations from rats to humans in all cases considered here make the simplifying assumption that the human exposures for which risk is to be predicted are low enough that only linear effects need be considered.

Time Scale. A quantitative issue in using the time-to-tumor model is the precise correspondence of time scales between rodent and human. The present analysis assumes that the reported median life span of the control rats in the study, 131.7 weeks, corresponds to the target human lifetime, 70 yr, at which the value of unit risk to humans is to be estimated, according to California guidelines (DHS 1985).

Dose metrics. For cumulative atmospheric exposure, the dose metric which is assumed to produce equal effects in rats and humans is the average lifetime equivalent rate of intake of particulate per body surface area. This follows the California guideline assumption. For the cumulative exposure to lung burden of diesel exhaust particles in the alveoli of the lung, the dose metric is mass per surface area of the alveoli. This metric assumes that the surface concentration of diesel exhaust particles is the appropriate measure of the carcinogenic agent.

Use of alveolar surface area as the denominator of the lung-burden metric is based on the general principle of using the concentration as near as possible to the active site to estimate the probability of toxic effects, assuming the diesel exhaust carcinogenesis occurs at the contact surface of the epithelium of the lung alveoli (Bond *et al*. 1989; Mauderly *et al*. 1987a). Ishinishi (1986a) reported that the main change in the alveolar region due to diesel exhaust exposure was accumulation of foci of carbon-laden macrophages in the alveoli. Infiltration of macrophages into the alveolar septa was also observed as a lesser effect. Morphological data from Parent (1992) show that alveolar surface area scales from rats to humans in the same ratio as does the number of exposed (epithelial) cells. Thus, lung burden per surface area represents the exposure concentration per DNA of exposed cells, as appears to be appropriate for purposes of scaling.
The rate of intake per body surface area. Equivalent atmospheric concentrations in humans are estimated from rat data using a scaling factor based on the assumption that equal values of atmospheric intake per body surface area produce equal risks. Thus, for equal risks, human and rat values of intake per body surface area are equated.

$$(EVX/A)_{rat} = (EVX/A)_{human}, \qquad (4)$$

where

E = fraction of inhaled mass retained in respiratory tract,

V = long term average inhalation rate, volumetric flow (m^3/day),
X = long-term average atmospheric concentration of diesel particulate ($\mu g/m^3$),
A = body surface area (cm^2).

Now at low concentrations the approximate equation of the additional (beyond background) risk in rats and humans is

$$(q_1 X)_{rat} = (q_1 X)_{human}, \qquad (5)$$

where

q_1 = unit risk ($(\mu g/m^3)^{-1}$).

Solving this equation for $(q_1)_{human}$ gives

$$(q_1)_{human} = X_{rat}/X_{human}\ (q_1)_{rat}. \qquad (6)$$

Thus, for a specified metric of dose equivalence, the ratio of exposure, X_{rat}/X_{human}, gives the scaling factor to apply in order to convert the unit risk in the rat, obtained above, to the unit risk in humans. The values of q_1 in this equation refer to different conditions. The q_1 for the rat refers to the 35 hr/wk of rat exposure, whereas the q_1 for the human refers to a continuous lifetime exposure.

Solving for the ratio of exposure concentrations in Equation 4 and using the resulting expression in Equation 6 then gives the scaling factor,

$(q_1)_{human}/(q_1)_{rat} =$

$$X_{rat}/X_{human} = (E_{tot}V/A)_{human}/(E_{tot}V/A)_{rat} \qquad (7)$$

Table G-5 provides values of the appropriate respiratory parameters V and E_{tot}. The table also gives values of W, and these values permit an allometric evaluation of the ratio for body surface area.

$$A_{human}/A_{rat} = (W_{human}/W_{rat})^{0.67} = (260)^{0.67} = 41.5. \qquad (8)$$

Inserting this ratio and the values from Table G-5 into the preceding equation gives a scaling factor of 2.2. In addition, an adjustment factor of 168/(hr/wk)/35(hr/wk) = 4.8 is required to multiply the $(q_1)_{rat}$ values resulting from a 35 hr/wk exposure to obtain the lifetime continuous equivalent average for humans.

Lung burden of diesel exhaust particles per alveolar surface area. Scaling the lung burden for rats and human starts with equating their respective lung burdens of particles per unit of alveolar surface area:

$$(m/S)_{rat} = (m/S)_{human}, \qquad (9)$$

where

m = average lifetime lung burden, mass of diesel exhaust particles in the alveoli (mg),
S = total alveolar surface area (m^2).

The conversion of the risk per lung burden in the rat to the risk per atmospheric concentration in the human proceeds by equating the risks expressed using those respective unit risks:

$$(q_1\ X)_{human} = (Q\ m)_{rat}, \qquad (10)$$

where

Q = unit risk in rats per lung burden (mg^{-1}).

Solving this equation for the unit risk for humans gives

$$(q_1)_{human} = (Q\ m)_{rat}/X_{human}. \qquad (11)$$

Using Equation 9 to eliminate m_{rat} from this equation gives the conversion,

$$(q_1)_{human} = (Q)_{rat}\ (m/X)_{human}\ S_{rat}/S_{human} \qquad (12)$$

At low exposures the lung burden per exposure concentration (m/X) can be derived from the mass balance of net intake into the alveolus, the net clearance from the alveolus and the exchange between regions of readily accessible particles and less accessible particles. See Table G-6a and Figure G-1.

$$(m/X) = (1 + k_{21}/k_{12})VE_{alv}/k_{01}, \qquad (13)$$

where

k_{01}, k_{12}, k_{21} are mass transfer coefficients (Table G-6b),
E_{alv} = efficiency of alveolar deposition of particles at low exposures.

[Note that this result only applies to the low exposures expected for ambient exposure to diesel exhaust and is used in this analysis only for the purpose of converting risk in terms of lung burden to risk per atmospheric concentration.]

For humans, taking the average of values in Table G-6 and using V = 20 m^3/d and E_{alv} = 0.15 from Table G-5 gives m/X = 1240 m^3. Thus, Equation 12 becomes

$$(q_1)_{human} = (Q)_{rat}\ 1240\ m^3/340 = (3.6\ m^3)\ (Q)_{rat}, \qquad (14)$$

again using Table G-5. Using lung burden, rather than exposure, there is no adjustment of this factor for exposure time.

In this evaluation, the values for alveolar surface area, S, were both determined by electron microscopy and are thus higher than values determined earlier by light microscopy. The appropriate value for E in this calculation is E_{alv} because the model uses a mass balance in the

alveolus. The values for clearance rate, R, require some recalculation from the parameters given in the literature, estimated assuming a two-component-exponential decay. Consistent with the assumption, alveolar clearance is represented by a two compartment model with one of the compartments acting as a storage compartment. Table G-6a gives the mathematical modeling relationships for the mass balance in the alveolus, and Table G-6b gives the parameters of the model. The value used for R in Table G-5 is then simply k_{01}, the coefficient for rate of transfer out of the alveolus, from Table G-6b.

G.2.7 RESULTING UCLS ON UNIT RISK FOR HUMANS

Table G-7 shows the results of converting the UCLs on unit risk for rats for the Mauderly *et al*. (1987a) data --the q_1* values in Table G-4 -- to UCLs on unit risk for humans. The risks predicted using the Weibull form of the multistage model are slightly greater than the risks predicted by the corresponding case for the Moolgavkar form. Also apparent in the table are the greater predictions using intake per body surface area compared to lung burden per alveolar surface area. The overall range of human predictions based on models is about 6-fold and ranges from 0.54×10^{-4} $(\mu g/m^3)^{-1}$, for the Moolgavkar form with scaling based on lung burden scaled per alveolar surface area, to 2.8×10^{-4} $(\mu g/m^3)^{-1}$ for the Weibull form based on intake rate per body surface area.

G.2.8 SOURCES OF UNCERTAINTY IN THE QUANTITATIVE RISK ESTIMATES BASED ON MAUDERLY *et al.* (1987) RAT BIOASSAY

Substantial uncertainties enter the calculation at many stages. These uncertainties could have a substantial effect on the numerical value of the risk estimates.

1. Use of all lung tumors except squamous cysts. The modeling calculations used all lung tumors reported by Mauderly *et al*. (1987a), except for squamous cysts. The individual tumor types identified for each affected animal were adenoma (6 animals), squamous cysts (13 animals) and squamous cell carcinoma or adenocarcinoma or both (23 animals). Thus, the total number of tumors in parentheses in Table G-1 is a mix of benign and malignant (patently cancerous) tumors. Adenomas are generally considered to have the potential to progress into malignant tumors. The progression potential of squamous cysts (or benign keratinizing cystic squamous cell tumors) is considerably more controversial. They have been described by various authors as being nonneoplastic (Mauderly *et al*., 1994), or as having the potential to progress to malignant tumors (Kittel *et al*., 1993). Some recent rat diesel exhaust carcinogenicity bioassays (Heinrich *et al*., 1995; Dasenbrock *et al*., 1996) have included squamous cysts in their total tumor incidence data. However, the squamous cysts appear so late in the lifetime of the rats that if they did have the potential to progress into a malignant tumor they would have little time to progress. Therefore, the squamous cysts were excluded from the final analysis due to their uncertain biological significance. The exclusion of squamous cysts had little effect on the linearized calculations. To test the possibility that the relatively large number of squamous forms at high exposures may be overwhelming the quantitative relationship at low exposures, further calculations of risk were made. These calculations showed that the dose-response for only

adenomas and adenocarcinomas together had a much greater linear component than did only squamous cysts and squamous cell carcinomas together and resulted in a predicted unit risk that was 30% less than that calculated for all tumors.

2. Selection of model for low-dose interpolation. The selection of the appropriate risk model for low dose interpolation is uncertain. In Table G-7 the range of results resulting from using even the most directly indicated models shows the consequences of that uncertainty.

Although considerable evidence suggests that carcinogenesis is a multistage process, the detailed nature of that process is uncertain; so mathematical models based on that process are uncertain and may vary for different carcinogens. Lacking compelling information to the contrary, the simplest multistage model appropriate for time-to-tumor data, the Weibull (in time) model, requires consideration.

For diesel exhaust the morphological and lung-burden information suggests that a cell proliferation effect may be occurring. To take this into account the analysis included the simplified Moolgavkar form of the multistage model. The calculations for the two-stage Moolgavkar model (Table G-4) returned an MLE result of zero for the value of coefficients, v_1 and v_2, which represent the effect of dose dependency on mutation. This result appears to reflect the predominance of cell proliferation effects in the high exposure groups and the uncertainty of interpolating the MLE values at low exposures. Use of the exact form of the Moolgavkar model with detailed time of exposure would give more precise predictions. The use of the simplified form introduces some uncertainty into the resulting predictions of risk.

The maximum values of the log-likelihood function in Table G-4 for each case allow some exploration of which forms and inputs fit the data better overall. The values of these maxima do not suggest a preference between the fits of the atmospheric-concentration input and the lung-burden input. The value of the log-likelihood function for the 2-stage Moolgavkar form is three units greater than that of the Weibull form, suggesting that the Moolgavkar form provides a better fit.

This suggestion follows from noting that both Equation 2 and Equation 3 are special cases of Bogen's general cell-proliferation form of model (Bogen, 1989). Each equation is obtained by assigning special values of parameters in the general form. With that recognition, the Moolgavkar model of Equation 3, which gives the greater maximum likelihood function (MLF) over the parameter space of the general form, may then be said to fit the data better in the sense of being more likely than the Weibull form of Equation 2.

For both model forms the ratios of 95% UCL to the MLE for q_1, namely $q_1^*/q(MLE)$ are approximately 5 for atmospheric concentration as a dose input and approximately 1.5 for lung burden as a dose input. These results show that a q_1 with lung burden as an input has a relatively narrow confidence interval compared to the value for atmospheric concentration.

The time-to-tumor analysis has an advantage over the quantal analysis using end of life data. Both types of analysis fit the data to multistage models, but the time-to-tumor analysis provides a

natural framework for incorporating data from serial sacrifices and makes use of the additional information on ages at examination.

A further uncertainty arises in the modeling of risks based on exposure to lung burden. The present analysis uses the assumption of a constant (average) level whereas lung burden rises with age, markedly at high exposure and older age. This effect would tend to increase estimates of risk based on rat data.

Unlike Armitage-Doll types of models, the models used do not take into account time weighting of exposure but only use simple cumulative exposures for atmospheric concentration and lung burden. The high degree of non-linearity exhibited by the bioassays suggests that the use of these Armitage-Doll models would be impractical because of the complex calculations which would require estimation of many parameters. Other possible models might also give more accurate low-dose extrapolation. For example, a model which could explicitly account for both the genotoxic effects that are likely to dominate the carcinogenesis at low exposures and the particle effects which are likely to dominate at high exposures can be contemplated. Such questions of model specification are a further source of uncertainty.

It is possible that other functional forms may fit the data with nearly the same likelihood values as the models selected. The analysis works with models that are considered to be the most plausible and is not concerned with a mathematically complete set of alternatives that have no previous justification. Use of such plausible models appears to give unit risks that are of the same order of magnitude. However, the mathematical alternatives are difficult to rule out and may be considered to be a source of uncertainty.

3. Range of unit risk estimates from the rodent data. This assessment focuses on 95% upper confidence limits (UCLs) for unit risk. These UCL values assure with 95% probability that if the model is appropriate, then the actual value of unit risk will be at or below the given value. The 95% UCL does not vary much with small changes in the data or the model details in the form of the model. In contrast the MLE values, which give the mode of the distribution of unit risk values, are very sensitive to small changes in the data or the model.

The magnitude of the range of carcinogenic responses among rat studies deserves mention here (see also Section G.2.4.1). It is true that at least one study (Brightwell *et al*., 1989) found significantly different carcinogenic responses among F344 rats at high doses compared with F344 rats from Mauderly *et al*. (1987a). However, the responses at the low end of the observable range were more consistent among studies and there is less than a 5-fold difference in the overall range of unit cancer risks calculated either from air concentration or lung burden of diesel exhaust from 5 studies using F344 or Wistar rats, as shown in Table G-3. Considering the differences in sample size and experimental design, the risk estimates that make up this range are very consistent.

There is possibly some inaccuracy in the calculation of upper confidence limits (UCLs) by likelihood methods due to some model parameters being constrained to be greater than zero. By using Monte Carlo methods as a standard, Portier and Hoel (1983) found that, when

misspecified, some highly constrained models -- pure linear or pure quadratic -- gave substantially incorrect estimates of unit risk, while more flexible models generally gave much better, though not completely accurate, results for a small number of test animals (150). The present UCLs have not been checked by Monte Carlo methods, but are believed to be essentially correct within a 2-fold factor. The number of animals in the primary study (Mauderly *et al*. 1987a) is about 900 and the models used have full flexibility except for the non-negativity constraint.

4. Extrapolation from animal to human. The difference between the predictions of risk using the California guideline dose metric of intake rate per body surface area and using the lung burden per alveolar surface area reflects some of the scaling uncertainty. Other scaling may also be feasible. For example, Hattis and Silver (1992) based their calculations on lung burden of diesel particulate per body weight. That metric has the effect of increasing human predictions of unit risk, based on lung burden, by 30% above using lung burden per surface area. Using values from Table G-5 in Equation 12, the ratio of surface area, 340, would be replaced by the ratio of body weights, 261. Data in Parent (1992) show that the alveolar surface-area denominator has the advantage of giving a ratio of human to rat that is nearly the same as the ratio for number of alveolar cells, reflecting the quantity of DNA at risk in those cells.

Predictions for sensitive individuals can depend on a host of factors. The work by Bohning *et al*. (1982) shows that smokers, for example, have a greatly impaired clearance. Calculations of Hattis and Silver (1992) predict that risk of diesel exhaust exposure in smokers substantially exceeds the risk in non-smokers.

Values of parameters in the scaling are subject to uncertainties due to biological variability. Table G-5 indicates a few aspects of the variability and uncertainty in the values used. An example of such uncertainty is in establishing the effective clearance rate R, both in animals and humans. The pharmacokinetic model used is a closed-storage form of a two-compartment model. This form reflects mechanistic information about the engulfment of diesel particles by macrophages, but there has been no definitive validation of this two-compartment model to characterize clearance. One compartment models yield 15 to 40% lower clearance rates in humans (Bohning *et al*., 1982; Bailey *et al*., 1982). For rats, Yu *et al*. (1991) reported clearance rates for one-compartment models that are about 75% lower than the present two-compartment result. Many of these results have been for high lung burdens, but one was conducted at very low concentrations (Wolff *et al*., 1987), as in the present calculations. The ten individuals used in each of the two human studies represent a rather narrow sample, only adult white males. In the present risk predictions based on lung burden, differences in effective clearance rate due to population heterogeneity will likely produce substantial variability and uncertainty in the estimate of the average risk.

Scaling to humans also entails uncertainty about how to proceed in view of background lung cancer rates being higher in human populations (smokers included) than in rats (Sielkin and Stevenson, 1994); see Section G.5.3, below.

The rat studies are not meant to model the responses of children. For instance, the rats in the Mauderly *et al*. study (1987a) began exposures at 17 weeks of age.

5. Use of the Rat as an Animal Model for Humans. Uncertainty exists in the use of the rat lung tumor data to estimate human cancer risk. As stated in Chapter 6, the mechanism of action by which diesel exhaust induces lung tumors in rats is not established. Data have been developed which support a nongenotoxic mechanism for rat lung carcinogenesis at high exposure concentrations. One proposed mechanism is that exposure to diesel exhaust particulate matter at high concentrations exceeds pulmonary clearance capabilities and causes chronic inflammation. This inflammation leads to macrophage and/or neutrophil-induced oxidative DNA damage resulting in mutations which are instrumental in the induction of lung tumors, and also to cell proliferation which may be mechanistically important to the promotion of the rat lung tumors. This mechanism may have an exposure threshold of action, suggesting that tumor induction due to this mechanism could also have a threshold. Although no mechanism has been established to account for the increased rates of lung tumors in diesel exhaust exposed workers, it has been proposed that any modeling of human cancer risk from rat lung tumor data should include an exposure threshold below which tumor induction would not occur. It has also been proposed that low level human exposure to diesel exhaust would not lead to the induction of lung tumors via the mechanism suggested for the rat. If so, the rat lung tumor data may not be a reliable predictor of any potential human cancer risk due to diesel exhaust inhalation. However, the genotoxicity due to the PAH and nitroPAH content of diesel exhaust may play a role in the induction of lung tumors in rats at lower levels of diesel exhaust. This mechanism would probably be relevant to human cancer risk, and would not be expected to have a threshold of action. It has been suggested that the PAHs and nitroPAHs adsorbed to the surface of diesel exhaust particulate matter may not be bioavailable. Green and Watson (1995) reviewed this subject and stated that "adsorption of organic molecules to carbonaceous particles enhances their penetration into the respiratory portions of the lungs but diminishes their bioavailability in proportion to the binding of the organic molecules and the agglomeration of the particle". However, data from a study by Kamens and Coe (1997) indicate that a number of the PAHs which condense on the diesel exhaust particulate matter carbon core may not be tightly physically bound to that carbon core. Additionally, Chapter 5 of this document describes the bioavailability of PAHs and nitro PAHs from diesel exhaust exposure in rats and humans, as well as increased lymphocyte DNA adducts in humans occupationally exposed to diesel exhaust. Chapter 6 notes the statement by Borm *et al*. (1997) that “an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particulates.” If so, low dose diesel exhaust exposure may result in levels of neutrophil influx which would not necessarily be detectable via histopathological examination as acute inflammation but which might be effective at amplifying any potential diesel exhaust genotoxic effect. WHO (1996) has noted that modeling of human cancer risk from rat lung tumor data should take into account the effects of both particles (carbon core) and extractable organic matter (PAHs, nitro PAHs).

Also, the proposed mechanism that exposure to diesel exhaust particulate matter at high concentrations exceeds pulmonary clearance capabilities and causes chronic inflammation includes the production of inflammatory cytokines and an increase in cell proliferation,

suggesting that this mechanism is either indirectly genotoxic or nongenotoxic. Gaylor and Zheng (1996) have stated that 1) a threshold dose is questionable if a nongenotoxic carcinogen acts via a cell receptor; 2) a nongenotoxic carcinogen that increases the cell proliferation rate by acting on the cell division rate is not likely to have a threshold dose; 3) dose response curves for cell proliferation and tumor incidence do not necessarily mimic each other. These increases in cell proliferation may be effected either by a stimulated increase in cell division or by an inhibition of apoptosis (programmed cell death).

As noted in Chapter 6, some parameters of the "particle overload" hypothesis are incompletely characterized. Evaluations of diesel exhaust-induced lung cell proliferation (Heinrich *et al*., 1995; Nikula *et al*., 1995; 1997) have used insensitive measures of cell proliferation (histopathological comparison to controls), making it premature to state that a true threshold of diesel exhaust-induced lung cell proliferation has been determined. Also, lung cell necrosis has not been noted in any of the rat diesel exhaust carcinogenicity studies. Uncertainties also exist regarding the magnitude and biological importance of particle overload for diesel exhaust-induced rat lung carcinogenicity. Indices of diesel exhaust-induced inflammation and cell proliferation do not correlate well with diesel exhaust-induced tumor incidence.

Hattis and Silver (1994) found that "there is continuing accumulation of diesel-derived dust in the lungs of rats throughout life, even at low doses", which was not predicted by models developed to represent diesel exhaust particulate matter accumulation under "overload" versus non-overload conditions. Additionally, they have found that at high diesel exhaust exposure levels, the increase in the ratio of internal diesel exhaust particulate matter burden to external exposure is not very large, being slightly larger than a factor of 2 at most, and state that "Although dust overloading is a real phenomenon, it is not a very large effect and thus would not be expected to give rise to dramatically lowered estimates of risk at low exposure levels."

The rat lung tumor data have not been shown to be mechanistically irrelevant to human cancer risk from diesel exhaust inhalation and, with these uncertainties acknowledged, provides information useful in the characterization of the potential magnitude of the human cancer risk associated with diesel exhaust exposure.

6. Ratio of dose from test chamber to environment. The exposure concentration of 3470 $\mu g/m^3$ particulate matter from diesel exhaust gave positive tumor results in rats in the test chamber (Mauderly *et al*. 1987). This exposure corresponds to an equivalent lifetime average concentration of 3470 $\mu g/m^3$ x 35 hr/168 hr = 730 $\mu g/m^3$. At that exposure in the experiment the average lung burden was 6220 μg (Table G-1), which gives an alveolar surface concentration of 15.5×10^3 $\mu g/m^2$. For a statewide environmental average atmospheric concentration of 3.7 $\mu g/m^3$ (Part A of this document), the corresponding lung burden is 4.6×10^3 mg, giving an alveolar surface concentration of 34 $\mu g/m^2$. See Table G-5 for the parameters needed for these conversions.

Based on these results for lung burden per alveolar surface area, the rounded ratio of the value for the test chamber to the value for the environment is 500. The ratio for the equivalent lifetime

average exposure concentrations is 200. Thus, the reduction in level needed to make the interpolation from laboratory to environmental values is quite substantial, though modest compared to many carcinogens. Still, in view of uncertainties that the model gives an accurate interpolation, this range indicates substantial uncertainty.

7. Diesel exhaust in animal test chamber as a representation of atmospheric diesel exhaust. The test engine used in the Mauderly *et al*. (1987a) study was a light-duty American automobile engine (1980 Model 5.7 L Oldsmobile V-8 engine) used in a U.S. Federal Test Procedure urban certification cycle. Composition of exhaust varies among other engines (1989) and other driving cycles, especially under road conditions. Different studies of light-duty engines have determined slightly different carcinogenic potencies (U.S. EPA 1994), possibly due in part to different study designs and/or exhaust compositions.

Heavy-duty engines dominate diesel exhaust on California roads (See Part A). Ishinishi *et al*. (1986a) studied the carcinogenicity of exhaust from both light-duty engines and heavy-duty engines. However, their results did not establish a clear difference in unit risk of the exhaust between the two engine types. Thus the present characterization of diesel exhaust unit risk is a useful estimate of the effect of diesel exhaust particulate in ambient air.

Any differences between the test exposures and ambient exposures to diesel exhaust owing to environmental transformation are not accounted for in this analysis. The complex composition of diesel exhaust and the many potential ways in which it could undergo atmospheric transformation are not adequately characterized.

Due to the above uncertainties and the availability of epidemiological data, OEHHA has focused on the risk estimates from epidemiological data for the final range of unit risks for humans.

G.2.9 COMPARISON TO PREVIOUS RISK ESTIMATES

Utilizing preliminary data from the study of Mauderly *et al*. (1986) in the quantal form of the multistage model (GLOBAL79), Albert and Chen (1986) presented preliminary estimates of unit risks in a conference proceedings. Those authors based risk values on each of the three tumor classes: (1) all tumors, (2) all except squamous cysts, (3) carcinomas only. Description of other procedures and adjustments is lacking, and the work has apparently not received peer review. Although these results have been cited in the literature, subsequent work has not replicated the very low risks they predicted. Thus, their results do not make an appropriate point of comparison here.

McClellan *et al*. (1989) estimated potency values based on rat inhalation studies (Mauderly *et al*., 1987a; Brightwell *et al*., 1986; Iwai *et al*., 1986; Ishinishi *et al*., 1986a). They used the logistic regression model, which has not been related to carcinogenic mechanisms, and their report does not describe detailed procedures and adjustments. So comparison with the present work does not appear to be appropriate.

For the unscaled unit risk of diesel exhaust carcinogenicity in the rat, Smith and Stayner (1990) obtained a maximum likelihood estimate (MLE) equivalent to a continuous lifetime unit risk of 1.8×10^{-4} $(\mu g/m^3)^{-1}$. They fit the Mauderly *et al*. (1987a) data to the Armitage-Doll form of the multistage model, which in this case is essentially the same as the Weibull form. Their scaling to humans, based on equivalence of average rate of particle deposition per body weight, gave a lower risk (1.8-fold less), but the MLE based on exposure was 5-fold higher than the present MLE result for the Weibull form, based on exposure. The main reason for this outcome appears to be that their form of the Armitage-Doll model allows only one stage of the process of carcinogenesis to be influenced by the applied carcinogen, implying that the exposure-response must be linear. Yet the analysis with the Weibull model gave a best fit for the exposure-response curve having a markedly steepening character. Thus, compared to the multistage model, their approach yielded a poorer fit and a substantially greater slope at low exposures.

Hattis and Silver (1992) reported results obtained by Smith and Stayner using the Mauderly *et al*. (1987a) data for lung burden, mostly increasing with time at a constant exposure. Lung burden per body weight was the dose equivalent for interspecies scaling. They reported an MLE for unit risk that is 4.5-fold higher than the one obtained using a constant average exposure, analogously to Smith and Stayner. The reason for the higher value of the MLE for continuous lifetime unit risk, 8.0×10^{-4} $(\mu g/m^3)^{-1}$, comes from the use of dose increasing with time in a model with only the first stage being influential in the five stages. This is because the early doses have the most effect in this case. Thus early doses will influence an effective dose, making it smaller than the average dose if the early doses are smaller than the late ones. Thus their unit risk is increased over the estimate based on the average.

The present work computes dose as the summed exposure to lung burden with time. The summation is on the approximate lifetime average value of lung burden. The present work uses lung burden per alveolar surface area for animal-to-human extrapolation, whereas Hattis and Silver used lung burden per body weight. The conversion factor for Hattis and Silver is analogous to the one specified in Eq (12), the ratio W_{rat}/W_{human} in place of S_{rat}/S_{human}. For their assumption of W = 0.35 kg, $W_{rat}/W_{human} = 200$. Also, their $(m/X)_{human} = 1470$ m^3 based on scaling presented in a figure in their paper, multiplying by body weight and adjusting for hours per week. Thus, their factor for converting risks per mg lung burden in rats to risks per $\mu g/m^3$ in humans is 7.35, multiplying the rat unit risk. This is about twice the value shown in Equation 14 (Section G.2.3).

In their risk assessment, Pepelko and Chen (1993) predicted human risks using data from Mauderly *et al*. (1987a), Ishinishi *et al*. (1986a), and Brightwell *et al*. (1986) in linearized multistage models. They predicted risk against inhaled particulate matter (mg/kg/day) and also against lung burden of particulate matter per alveolar surface area (mg/cm^2) for each study. They calculated lung burden using a dosimetry model of Yu *et al*. (1990) and Yu *et al*. (1991). For the Mauderly *et al*. (1987a) data, a time-to-tumor analysis with the Weibull form of the multistage model gave 8.9×10^{-5} $(\mu g/m^3)^{-1}$ and 1.0×10^{-5} $(\mu g/m^3)^{-1}$, respectively for the two values of lifetime unit risk, scaled to humans. The other two sets of data used only quantal analysis, giving values that ranged from 1.0×10^{-5} $(\mu g/m^3)^{-1}$ to 2.4×10^{-4} $(\mu g/m^3)^{-1}$.

G.3 COMPARATIVE POTENCY ESTIMATES

Before epidemiological or animal studies provided strong quantitative relationships between carcinogenicity and diesel exhaust, the "comparative potency" method was utilized to assess quantitatively the carcinogenic potential of diesel exhaust. In the comparative potency method, the risk of a suspect carcinogen (e.g. diesel exhaust) is estimated by comparison to a known carcinogen (e.g. coke oven emissions) according to the following equation (Albert *et al*., 1983; Lewtas *et al*., 1985):

$$\text{Estimated human risk}_{(\text{diesel})} = \text{human risk}_{(\text{coke oven})} \times [\text{bioassay potency}_{(\text{diesel})}/\text{bioassay potency}_{(\text{coke oven})}]$$

The ratio in the brackets expressing the relative bioassay potency is evaluated as the ratio of the slopes of the dose responses from the same in vitro or in vivo bioassay.

Using potency data from soluble extracts of the particulate matter resulting from the two different processes, two investigators calculated unit risks, reported here as central tendencies. Albert *et al*. (1983) estimated lifetime cancer risk from inhalation of 1 $\mu g/m^3$ diesel particulate to range from 2.0×10^{-5} to 3.5×10^{-5}, based on the average mouse skin tumor initiation activity. With the same general data, Harris (1983) estimated a yearly relative cancer risk of 3.5×10^{-5} $(\mu g/m^3 \times \text{years})^{-1}$. Using this slope in the modified California life table gives 9.6×10^{-5} $(\mu g/m^3)^{-1}$. Calculations of McClellan *et al*. (1989) and Mauderly (1992) translated Harris' result to a continuous lifetime unit risk of 7.6×10^{-5} $(\mu g/m^3)^{-1}$. Cuddihy and McClellan (1983) and Cuddihy *et al*. (1984) furnished similar analyses.

Although near some of the lower risks calculated above from rat data, the comparative estimates of unit risk based on the assumption of carcinogenic activity only in the soluble organic fraction of the diesel exhaust are about ten-fold lower than the predictions of human risk from the occupational studies. The comparative estimates should not be expected to be precise, in part because of the variable nature of the emissions of coke ovens and the paucity of historical measurement. Nevertheless, this difference between human observations and comparative estimates appears to be consistent with the present results. Some of the difference between the comparative results and the direct results is due to the use of a central tendency for the comparative potency method, thus giving a lower value than the 95% UCL. The remainder of the difference is consistent with the carbon core of the particle having the predominant quantitative influence on risk and the soluble extract having much less effect. Thus, this comparison provides some support for estimating risk of diesel exhaust as a whole, without considering the carcinogenic contribution of constituents of the exhaust.

G.4 ISSUES PERTAINING TO QUANTITATIVE RISK ASSESSMENT USING BOTH HUMAN AND ANIMAL DATA

G.4.1 EFFECT OF COMPOSITION OF DIESEL EXHAUST

The effect of the variable composition of diesel exhaust is uncertain. The present analysis assumes that carcinogenesis due to diesel exhaust is measured appropriately by the particulate matter fraction. With this assumption, the results of the rat bioassay in a model give predictions of human risk for which the bottom of the range is 20-fold less than the bottom of the range based on epidemiological results for railroad workers. For the top of the ranges the spread is 7-fold. Some of this difference could be due to the difference in the composition of diesel emissions between the automobile engines used in the Mauderly *et al*. (1987a) rat study and the large diesel engines used in the railroads. Although one study directly compared the carcinogenicity of diesel exhaust from an automobile engine to that of a truck engine and did not find any statistically significant difference (Ishinishi *et al*. 1986b), the difference between fuels and engines in that laboratory study may not have been as great as the difference between the automobile fuels and engines generally used in the laboratory studies and the fuels and engines of the studies on railroad workers.

G.4.2 OTHER SOURCES OF DIFFERENCE BETWEEN RESULTS OF RATS AND HUMANS

The reason for the apparent difference between rat-based and human-based estimates of cancer risk of diesel exhaust is not fully understood. Other effects that may contribute to an explanation for the difference may include the following: (1) Humans may simply be more sensitive than rodents to the effect of diesel exhaust on lung cancer. (2) The effects of smoking and other air pollutants may contribute to a greater effect of diesel particles in causing tumors in humans than would occur without smoking, thus leading to elevated predictions of risk for non-smokers. (3) Consideration of the higher background rate of lung cancer in humans than in the rats of the study may lead to increased human predictions of risk from using the rat data. Thus it appears that finding reliable ways of taking these effects into account quantitatively could close the range of the predictions based on animal data and the corresponding predictions based on human data. Such an outcome would tend to lower the prediction of risk based on human data. The assessment does not now calculate risks for specially sensitive humans, such as those with chronically impaired lung clearance, which may be the result of respiratory disease. Finding reliable ways of taking such conditions into account would also provide a more comprehensive risk assessment.

G.4.3 THE EFFECT OF SMOKING

When corrected for smoking, the present ranges of the human-based and rat-based estimates of lung cancer risk from diesel exhaust become overlapping. Such a correction would put the two predictions into the same smoking status -- starting with nonsmoking. On the assumption of a multiplicative model with no interaction of the effects of smoking and diesel exhaust, as found in the Garshick case-control study (1987a), such a correction can readily be approximated. As

shown in Table 7-4 this correction converts the baseline California population incidence rate for lung cancer, used to calculate the above unit risks, to a non-smoking incidence rate. The result is that the human-based unit risks would be reduced approximately 7-fold, bringing the human-based range within the rat-based range. Nevertheless, the human-based range, given above as 2×10^{-4} to 2×10^{-3} (lifetime-$\mu g/m^3)^{-1}$, is appropriate for making decisions to protect the health of Californians. A substantial proportion of this population smokes, as reflected in the lifetime risk for lung cancer, 0.025, computed for the California population in Table D-1 and used to calculate these unit risks. This value is 7-fold higher than for nonsmokers. Accordingly, the rat-based risks would need to be increased 7-fold in order to be used to make appropriate predictions for the California population.

G.5 CONCLUSION

The rat bioassay of Mauderly *et al*. (1987a) and several other studies would provide a basis for estimating human risks if no reliable human estimates were available. The results of Mauderly *et al*. using two different models of carcinogenicity and two measures of exposure, yield a lowest calculated 95% UCL for unit risk of 0.5×10^{-4} $(\mu g/m^3)^{-1}$. This value is based on the Moolgavkar cell-proliferation model using rat lung burden extrapolated to human lung burden per alveolar surface area. The highest 95% UCL for unit risk value from the Mauderly *et al*. study was 3×10^{-4} $(\mu g/m^3)^{-1}$ using the Weibull model with rat inhalation exposure levels as input, and extrapolation based on intake per body surface area.

The range of 95% UCL for risk from the five rat studies described in Section G.2 , after fitting the rat data and scaling the results to humans, is 1×10^{-5} to 3×10^{-4} $(\mu g/m^3)^{-1}$. A geometric mean unit risk estimate from the rat studies determined in this document and in U.S. EPA (1994) is 6×10^{-5} (lifetime-$\mu g/m^3)^{-1}$. The UCLs based on rat data are slightly within or below the bottom of the range of risks from human data and the bottoms of the respective ranges are 20-fold apart. This divergence may be due to a greater sensitivity of humans, to underestimates of exposure in the human studies, to uncertainty regarding the appropriate way in which to calculate scaling from rodents to humans or to other factors.

The uncertainty in the scaling of rat predictions to humans is substantial. The scaling of such important characteristics as clearance rates, the presence or absence of a threshold for onset of carcinogenic effects, or the possible presence of multiple carcinogenic mechanisms all contribute to the uncertainty. The present lack of knowledge about how the carbon core of the diesel exhaust particle contributes to carcinogenicity also adds to the uncertainty about the scaling from rats to humans. Because of these uncertainties and the availability of epidemiological data, OEHHA has decided to use risk estimates based on human data for the final range of risks to humans (see Table 7-10).

The strengths and weaknesses of calculating population risks using the human studies (Garshick *et al*. 1988, Garshick *et al*. 1987a) and the animal bioassays (Mauderly *et al*. 1987a, Brightwell *et al*. 1989, Heinrich *et al*. 1995, Ishinishi *et al*. 1986a, Nikula *et al*. 1995) are presented in Table 7-6. This summary is based on the issues discussed above, especially in Section G.2.8 of

this appendix and Section 7.2. As discussed above (Section G.4.3), an approximate correction for smoking would raise the rat-based unit risks into near coincidence with the human-based unit risks as applied to the California population.

On balance, the human data lend more confidence in the prediction of human risks than the data from the rat studies because of the uncertainties of extrapolating from rats to humans, especially in the context of a substantial particle effect. These uncertainties in this species extrapolation appear to outweigh the uncertainties of using the epidemiological results -- uncertainties of the actual exposure history, the modeling and data selection. The uncertainty in the extrapolation from animal data is difficult to quantify, but is likely to be much greater than in using human data.

Table G-1 Incidence of Lung Tumors in F-344 Rats (Mauderly *et al.* 1987a).

Exposure Concentration[a] (mg/m^3)	Average Excess Lung Burden[b] (mg)	Number of Animals[c]	Number with Tumors[d]
0.01	0	230	2
0.35	0.37	223	3
3.47	6.22	222	6 (8)[e]
7.08	12.42	227	18 (29)[e]

[a] Exposure started at beginning of the 18th week and lasted until the 151st week.

[b] Computed from the study by linear interpolation and extrapolation as used by Hattis and Silver(1992)

[c] Males and females combined.

[d] No distinction between sacrifice and natural death. All tumors considered incidental. Week of age at detection of tumors (Multiple animals in parentheses):

94,96; 119, 140, 146; 99, 132, 135, 144, 145(2), 146, 150; 109, 113, 117, 121, 123, 129, 131, 133, 137(2), 138, 139, 140, 143, 145, 146(2), 150(11), 151

[e] Includes total tumors without squamous cysts. Total tumors with squamous cysts are shown in parentheses.

Table G-2 Lung Tumors in Rats Following Inhalation of Diesel Particles.

Study	Strain (gender)	Exposure schedule	*Time-Weighted Average Concentration (mg/m^3)*	Ambient Exposure concentration (mg/m^3)	Lung tumor incidence *Lung squamous cysts included*	Lung tumor incidence *Lung squamous cysts not included*
Brightwell *et al*. (1989)	F344 (female and male)	16 h/d, 5 d/wk	*0* *0.33* *1.05* *3.14*	0 0.7 2.2 6.6		4/250 1/112 14/112** 55/111**
Heinrich *et al*. (1995)	Wistar (female)	18 h/d, 5 d/wk, 30 mo	*0* *0.45* *1.34* *3.74*	0 0.84 *2.5* *6.98*	1/217 0/198 11/200** 22/100**	
Ishinishi *et al*. (1986a)	F344 (female and male)	16 h/d, 6 d/wk, 30 mo	*0.26* *0.55* *1.05* *2.13*	0 0.46 0.96 1.84 3.72		1/123 1/123 0/125 4/123 8/124*
Mauderly *et al*. (1987a)	F344 (female and male)	7 h/d, 5 d/wk	*0.00* *0.07* *0.72* *1.48*	*0.01* 0.35 *3.47* 7.08	[2/230] [3/223] [8/222] [29/227]	2/230 3/223 6/222 18/227**
Nikula *et al*. (1995)	F344 (female and male)	16 h/d, 6 d/wk, 24 mo	*0* *1.43* *3.71*	0 2.5 6.5		3/214 13/210** 38/212**

* Significantly different from controls ($p < 0.05$) using a one-sided Fisher's exact test.
** Significantly different from controls ($p < 0.01$).

Table G-3 Rat and Human Unit Cancer Risk Estimates from Rat Studies.

Study	Rat unit cancer risk based on lung burden (per µg/m^2 alveolar surface area)[a]		Human unit cancer risk based on concentration (per µg/m^3)[b]		Human unit cancer risk based on lung burden (per µg/m^3)[c]	
	MLE	95% UCL	MLE	95% UCL	MLE	95% UCL
Brightwell *et al*. (1989)	1.7×10^{-2}	3.9×10^{-2}	7.2×10^{-5}	1.6×10^{-4} [d]	3.3×10^{-5}	7.7×10^{-5}
Heinrich *et al*. (1995)	5.2×10^{-3}	1.4×10^{-2}	2.9×10^{-5}	7.6×10^{-5} [d]	1.0×10^{-5}	2.7×10^{-5}
Ishinishi *et al*. (1986a)	8.4×10^{-4}	8.3×10^{-3}	5.0×10^{-6}	4.9×10^{-5} [d]	1.6×10^{-6}	1.6×10^{-5}
Mauderly *et al*. (1987a)	4.5×10^{-3}	1.4×10^{-2}	2.9×10^{-5}	9.0×10^{-5} [e]	8.8×10^{-6}	2.8×10^{-5}
Nikula *et al*. (1995)	5.3×10^{-3}	1.8×10^{-2}	2.9×10^{-5}	9.9×10^{-5} [e]	1.0×10^{-5}	3.5×10^{-5}
Geometric mean	4.5×10^{-3}	1.6×10^{-2}	2.4×10^{-5}	8.9×10^{-5}	8.8×10^{-6}	3.2×10^{-5}
Arithmetic mean	6.6×10^{-3}	1.9×10^{-2}	3.3×10^{-5}	9.6×10^{-5}	1.3×10^{-5}	3.6×10^{-5}
Median	5.2×10^{-3}	1.4×10^{-2}	2.9×10^{-5}	9.0×10^{-5}	1.0×10^{-5}	2.8×10^{-5}

[a] Based on LMS modeling of average lifetime ambient concentration vs. lung tumor incidence.

[b] Based on LMS modeling of average lifetime lung burden vs. lung tumor incidence.

[c] Based on interspecies scaling from ambient concentration derived rat unit cancer risk using relative pulmonary intake rate and body surface area.

[d] Includes squamous cell cysts.

[e] Excludes squamous cell cysts.

LMS modeling of Mauderly *et al*. (1987a) tumor incidence data inclusive of squamous cell cysts results in MLE = 3.9×10^{-5} and 95% UCL = 9.9×10^{-5} based on concentration.

Table G-4 Numerical Values Obtained Using Multistage Models for Rats, Based on Mauderly *et al.*, 1987[a] (without squamous cysts).

	For Atmospheric Concentration in $\mu g/m^3$	For Lung Burden in μg
Weibull[b]		
r_0	3.21×10^{-12}	3.27×10^{-12}
r_1	7.46×10^{-13}	3.35×10^{-13}
r_2	0	0
r_3	6.66×10^{-14}	1.28×10^{-14}
c	4.651	4.652
Maximum log-likelihood	-106.76	-106.78
$\mathbf{q_1^*}$	$\mathbf{2.6 \times 10^{-5}}$	$\mathbf{1.4 \times 10^{-5}}$
q_1 (MLE)	5.3×10^{-6}	2.4×10^{-6}
Moolgavkar[c]		
v_0	7.62×10^{-7}	7.77×10^{-7}
v_1	0	0
v_2	0	0
v_3	1.67×10^{-2}	1.64×10^{-2}
v_4	5.15×10^{-6}	2.94×10^{-6}
v_5	1	1
Maximum log-likelihood	-103.89	-103.93
$\mathbf{q_1^*}$	$\mathbf{9.0 \times 10^{-6}}$	$\mathbf{5.1 \times 10^{-6}}$
q_1 (MLE)	5.0×10^{-6}	2.8×10^{-6}

[a] Models are of the time-to-tumor type with time in weeks. All values in the table are the unadjusted unscaled computer outputs for the rat using the TOXRISK program. All values are maximum likelihood estimates except for q_1^*. The q_1^* values (in bold) are the 95% UCLs for the unit risk, q_1, and are for the median lifetime of the rats in the study: 131.7 weeks, rounded to 132 weeks.

[b] The 3-stage Weibull (in time) model was

Table G-4. Numerical Values Obtained Using Multistage Models for Rats, Based on Mauderly et al., 1987[a] (without squamous cysts) (*continuation*).

$P(d) = 1-\exp(\{-r_0-r_1h(d-d_0)-r_2[h(d-d_0)]^2-r_3[h(d-d_0)]^3\}\ [h(t-s_1)]^c)$,
where
r_i = constant coefficient, to be determined (i=0,1,2,3),
d = dose or exposure concentration,
d_0 = threshold value,
c = constant exponent
s_1 = latency time,
$h(x) = \lceil 0,\ x < 0,$
$\lfloor x,\ x > 0.$

[c] The simplified Moolgavkar model was used with a cell proliferation function that is linear in exposure or dose.

$P(d,t) - 1- \exp[\ -(F/G^2)\ \{\exp[Gh(t-s_1)]-Gh(t-s_1)-1\}\ ]$
where
$F = v_0 +v_1h(d-d_1) + v_2[h(d-d_1)]^2$,
$G = v_3 + v_4[h(d-d_1)]^v5$ = cell proliferation function,
v_1 = constant,
d_0 = dose threshold in G,
d_1 = dose threshold in F.

The computer program returned estimates of zero latency and of zero dose threshold in both F and G for both measures of dose.

[d] These values of q_1^* require multiplication by 168 (hr/wk)/35(hr/wk) = 4.8 to adjust the risk estimates in $(\mu g/m^3)^{-1}$ to full-time continuous exposure. The resulting values give 95% UCL for unit risk for rats. The human values then require selection of a scaling factor.

[e] These values of q_1^* require multiplication by 3.6 m^3 (Eq. 15) in order to convert the risk estimate (in units of μg^{-1}) to units of $(\mu g/m^3)^{-1}$. This conversion gives the value of 95% UCL for unit risk for continuous lifetime human exposure. The human values then require selection of a scaling factor.

[f] q_1 (MLE) = 4.8 r_1 T^c, where T = the animal lifetime, 132 wk, as in footnote a.

Table G-5 Parameters Used in Scaling the Effect of Exhaust Particles[a].

Parameter	Dimensions	Rat (F-344)	Human
W^b	kg	0.27^h	70^i
$E_{tot}{}^c$	-	0.15^j	0.23^k
$E_{alv}{}^c$		$0.11(0.10, 0.12)^j$	$0.15(0.11, 0.20)^k$
V^d	m^3/d	0.34^h	20^i
$R_{10}{}^e$	d^{-1}	0.059^l	0.0028^m
$(m/X)_0{}^f$	m^3	1.6 ± 1.5	1240 ± 33
S^g	m^2	0.40 ± 0.014^n	135 ± 12^o

a. Values in parentheses represent the range of bulk of data. Values with ± give the standard error of the mean.
b. W = average body mass
c. E = deposition efficiency: alv = alveolar, tot = total respiratory.
d. V = volumetric inhalation rate, long-term average.
e. R_{10} = long term clearance rate from alveolar region from Tables G-6.
f. $(m/X)_0 = (1 + k_{21}/k_{12})\ EV/k_{01}$ = normalized lung burden at low exposure, for animal experiment and human continuous exposure, as in Table G-6a. This value is obtained by averaging the values obtained for each of the individuals in Table G-6b.
g. Alveolar surface area.
h. Mauderly *et al*. (1986): From nose-only exposure studies.
i. Human standard value.
j. Chan *et al*. (1981), Wolff *et al*. (1984), Yu *et al.* (1987).
k. Yu *et al.* (1990, 1991).
l. Wolff (1987) data with no diesel exposure and low exposure to tracer particles. Excludes first day.
m. Bohning *et al*. (1982), Bailey *et al*. (1982). Excludes first week.
n. Pinkerton *et al*. (1982), average for F-344 rat.
o. Gehr *et al*. (1987), adjusted by the ratio of standard body weight to observed body weight x (70/74).

Table G-6a Two-compartment Model with One Compartment for Storage: Governing Equations.

From Fig. 7-1 the governing equations at low concentrations are:

$$\frac{dm_1}{dt} = I - k_{21}m_1 + k_{12}m_2 - k_{01}m_1$$

$$\frac{dm_2}{dt} = k_{21}m_1 - k_{12}m_2,$$

where m_1 and m_2 are the masses (mg) in compartments 1 and 2 and

t = time (d)
I = intake rate (mg^3/d),
k_{21}, k_{12}, k_{01}, are clearance rate constants (d^{-1}).

For I = 0 the solution that has $m_1 = m_{10}$ at 0 is (Atkins, 1969)

$$m_1 = m_{10} [b_1 \exp(R_{10}t) + (1-b_1)\exp(R_{20}t))]$$

where the parameters reported in the studies give b_1 and

$$2R_{10} = - (k_{12}+k_{21}+k_{01}) - [(k_{12}+k_{21}+k_{01})^2 - 4k_{01}k_{12}]^{½},$$
$$2R_{20} = - (k_{12}+k_{21}+k_{01}) + [(k_{12}+k_{21}+k_{01})^2 - 4k_{01}k_{12}]^{½}.$$

Then, solving in turn for the clearance rate constants

$$k_{12} = - b_1 R_{20} - (1-b_1) R_{10} ,$$
$$k_{01} = R_{10}R_{20} / k_{12} ,$$
$$k_{21} = -k_{01} -k_{21} -R_{10} -R_{20} .$$

At steady state, the left-hand sides of the governing equations both become zero. Then, taking the difference between the two gives $I = k_{01}m_1$, which corresponds to Eq. 13. Also, $m_2 = m_1 k_{21} / k_{12}$. Expressing the intake rate as XVE_{alv} gives

$$(m_1/X) = VE_{alv} / k_{01},$$

where

X = atmospheric concentration of particles, (mg/m^3),
E_{alv} = efficiency of alveolar deposition (-),
V = inhalation rate (m^3/d).

Therefore, the normalized lung burden becomes

$$(m_1+m_2) / X = (m/X)_0 = (1+k_{21}/k_{12}) VE_{alv} / k_{01}.$$

Table G-6b Two Compartment Model with One Compartment for Storage: Parameters.

Study[a]/particle	b_1[b]	T_1[c]	T_2[d]	k_{12}[e]	k_{01}[e]	k_{21}[e]	$(m/X)_0$[e]
		(d)	(d)	(1/d)	(1/d)	(1/d)	(m^3)
Rat[f]							
Wolff *et al*. 87 Cs-FAP[g]	0.810	1.6	79	0.0894	0.0425	0.3101	3.93
Ga_2O_3	0.620	0.9	36	0.03046	0.0487	0.4361	1.87
	0.750	1.0	25	0.1941	0.090	0.4278	1.21
(overall)[h]	0.550	0.9	45	0.3550	0.0334	0.3971	2.37
Human[i]	0.630	0.9	37	0.2968	0.0486	0.4435	1.92
Bailey *et al*.85[h] FAP[j]	0.638	0.9	36	0.2876	0.0574	0.4261	1.62
Bailey *et al*. 82 FAP[j]	0.140	40.0	350	0.0152	0.0023	0.0019	1490
	0.083	17.0	309	0.0376	0.0024	0.0030	1331
	0.120	32.0	239	0.0194	0.0032	0.0019	1081
	0.070	10.0	390	0.0646	0.0019	0.0046	1685
	0.030	10.0	393	0.0673	0.0018	0.0020	1700
	0.100	24.0	315	0.0262	0.0024	0.0024	1335
	0.040	10.0	291	0.0666	0.0025	0.0026	1258
(overall)[h]	0.072	17.2	326	0.0488	0.0024	0.0027	1334
Bohning *et al*. 82	0.270	36.0	292	0.0147	0.0031	0.0038	1215
Latex	0.160	7.0	232	0.0837	0.0035	0.0148	999
	0.370	32.0	280	0.0146	0.0037	0.0059	1144
	0.380	45.0	275	0.0105	0.0037	0.0037	1099
	0.250	75.0	362	0.0074	0.0024	0.0014	1487
	0.270	20.0	313	0.0259	0.0030	0.0080	1325
(overall)[h]	0.286	35.0	292	0.0284	0.0033	0.0068	1142

[a] These studies have the lowest lung burdens of any studies giving a full two-exponential result. Note that $(m/X)_0$ is the prediction of normalized lung burden at low exposure.

[b] Parameter reported in the study with normalized lung burden given by $b_1 \exp(-\mathrm{Ln}(2)\, t/T_1) + (1-b_1) \exp(-\mathrm{Ln}(2)\, t/T_2)$

[c] Smaller observed time constant. Note that $R_{10} = \mathrm{Ln}(2)/T_1$.

[d] Larger observed time constant. Note that $R_{20} = \mathrm{Ln}(2)/T_1$.

[e] Parameters calculated for two compartment model in Table 7-4a.

[f] Values used in modeling clearance at low exposures in rats.

[g] Cesium-tagged fused aluminum silicate particles.

[h] Means except for last column, which is a prediction based on those means to the left.

[i] Values used in modeling average human clearance.

[j] Fused aluminum silicate particles.

Table G-7 Human UCLs for Unit Risk for Diesel Exhaust Predicted from Mauderly *et al*. Rat Data [a] [x 10^{-4} ($\mu g/m^3$)$^{-1}$].

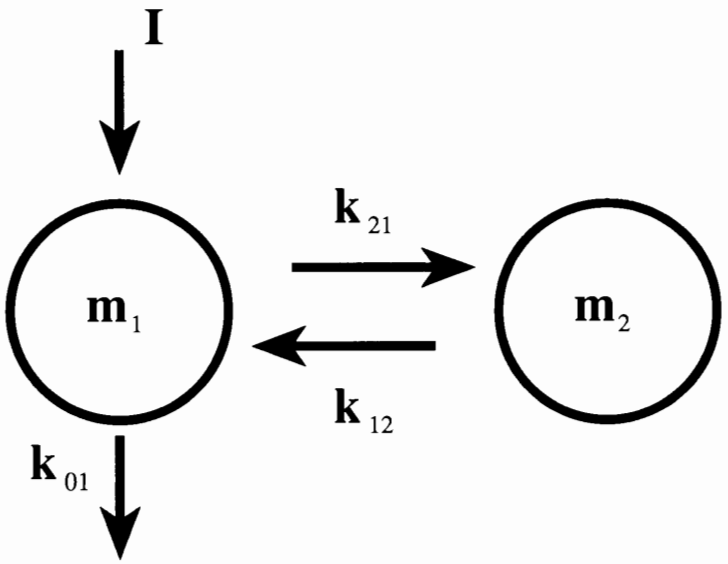
Risk Model:	Dosimetric Equivalence		
	Intake/ Body Surface[b]	Air Concentration	Lung Burden/ Lung Surface[c]
Weibull:	2.8	1.30	1.5
Moolgavkar:	0.95	0.43	0.54

[a] Upper 95% confidence limits on unit risk, predicted for mean lifetime of the rats in the study, 132 weeks. Squamous cysts were not included in the analyses.

[b] Results of multiplying the rat risk from atmospheric concentration by a scaling factor of 2.2, based upon equivalence of uptake rates per body area.

[c] Calculated for average lung burden and then expressed with reference to atmospheric concentration for low exposures.

Figure G-1 Two compartment model with one compartment open to the environment.



This diagram gives the flow pathways (arrows) and storage compartments (large circles) for the two-compartment model (after Atkins, 1969) used in the case of low concentrations to calculate the time course of lung burden (the sum of the masses, m1 + m2) of diesel soot in the alveolar region of the lungs. In physical terms the two "compartments" represent states of the particles and not local regions. The compartemnts in this case represent (1) the particles that are relatively unbound in the alveoli throughout the lung and (2) the particles that are relatively bound in the alveoli. I is the overall rage of intake of particles into the lungs and the k's are the various transfer coefficients between the compartments.