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HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST PARTICLES[1]

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INTRODUCTION

The health effects of diesel exhaust (DE) have been a focus of intensive research during the last decade. Increased use of diesel engines in light-duty passenger cars and vans stimulated this research. These vehicles are used extensively in populated areas and thus could increase the exposure of urban residents to DE.

Diesel engine–powered vehicles emit more oxides of nitrogen and some 30 to 100 times more particles than do gasoline engines with contemporary emission-control devices. The small size of diesel exhaust particles (DEP) makes them readily respirable, which raises concern for their health effects. As early as 1955, Kotin et al (1) evaluated the chemical composition of diesel exhaust particle extracts (DEPE) and demonstrated their carcinogenicity in mouse-skin-painting studies. However, little additional research was done on DE until the mid-1970s, when advances in biology provided improved methods such as the Ames *Salmonella typhimurium* assay (2) for detecting mutagenicity and, potentially, carcinogenicity. In 1977 the US Environmental Protection Agency issued a precautionary notice (3) reporting that DEPE were mutagenic in bacterial assays. These findings were subsequently published by Huisingh et al (4). This observed mutagenicity triggered a major research effort on the health effects of DE. The results of this research have been the topic of several symposia and reviews (5–11), and are briefly reviewed in this article.

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PHYSICAL AND CHEMICAL CHARACTERISTICS

Shape, Size, and Surface Area

DEP are chain aggregates of very small, spherical primary particles. Under the microscope they appear as chains of beads or clusters of grapes. The aggregates have a mass median diameter of a few tenths of a micrometer, and the primary particles have a diameter of 10–80 nm (12, 13). The specific surface area of DEP is high and strongly dependent on the outgassing temperature, ranging from $\cong$ 10 m^2 per g at 25°C to $\cong$ 100 m^3 per g at 450°C (14). The high surface area indicates the potential for DEP to absorb large amounts of vapor-phase organic compounds.

Chemical Composition

The DEP consist primarily of carbon, hydrogen, oxygen, and nitrogen. They also contain trace quantities of sulfur, zinc, phosphorus, calcium, iron, silicon, and chromium, with nonextractable and extractable fractions (15). The nonextractable, or dry soot, fraction resembles carbon black. The extractable fraction (5–50%) is removed with solvents, such as dichloromethane or benzcne ethanol, using ultrasonication or continuous (Soxhlet) extraction of DEP collected on filters. The relative amount of extractable vs nonextractable material depends upon factors including engine type and condition, fuel composition, and load (16). For some engines the carbon in DEPE originates primarily from the fuel, whereas in other cases the lubricating oil is a major source of the carbon (17).

The chemical composition of DEPE has been extensively investigated (18–20). Because of interest in the biological activity of DEPE, much of the characterization work has involved bioassay-directed chemical analysis aimed to identify specific chemical classes and compounds responsible for biological activity. This approach uses liquid chromatography or liquid–liquid separation procedures to fractionate the compounds according to chemical functional groups. High-pressure liquid chromatography may be used to separate the DEPE into nonpolar, moderately polar, and polar fractions. Most of the mass is in the nonpolar fraction, which includes aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH), and higher molecular weight, bridged, and methylated PAH. The moderately polar fraction contains PAH ketones, aldehydes, quinones, and acid anhydrides as well as hydroxy- and nitro-PAH. The polar fraction contains PAH carboxylic acids and other oxygenated PAH.

When assayed with *S. typhimurium* strain 98, most of the mutagenic activity is found in the moderately polar fraction, less in the polar fraction, and very little in the nonpolar fraction. DEP probably contains over 1000 compounds, more than 100 of which have been specifically identified. Nitroaromatic compounds, for example, are major contributors to the

mutagenicity observed in the *S. typhimurium* assay in the absence of S-9, the liver enzyme fraction (21, 22). This observation has given impetus to their identification. Paputa-Peck et al (23) reported positive identification of 15, and tentative identification of 45, nitro-PAH, including naphthalenes, biphenyls, fluorenes, anthracenes, and pyrenes. Some investigators have suggested that a few individual compounds be considered "model compounds" to facilitate the study of exhaust and the development of quantitative measures of exposure and risk (19).

Pitts et al (24) observed that nitro-PAH could be formed during sampling of ambient air. This finding, and subsequent observations by Lee et al (25), triggered debate over the role of sampling artifacts in forming nitroaromatics during collection of DEP. Schuetzle (18) and Schuetzle & Perez (26) presented convincing evidence that little of the 1-nitropyrene measured in DEPE is formed during sampling; the majority of the nitro-PAH is formed in the engine and/or tailpipe.

SHORT-TERM IN VITRO AND ANIMAL STUDIES

Activity in Short-Term Bioassays

A number of papers on the effects of DEPE in short-term bioassays are included in recent symposia (7, 8, 11, 27). The review of Lewtas & Williams (27) is especially notable. It details the utility of a battery of assays in predicting carcinogenic potency. Assays in several strains of *S. typhimurium* suggest that the mutagens cause frameshift, but not base-substitution, mutations in the bacteria (4). The response occurred without an S-9 fraction, indicating that an exogenous source of promutagen activation was unnecessary. Indeed, with the exception of strain TA-1538 the response to DEPE was usually decreased by adding S-9 (28). Clark & Vigil (29) demonstrated that enzyme preparations from lung also decreased the mutagenicity of DEPE. They observed a similar decrease with serum and albumin, which suggested that the reduced effect might be due to nonspecific binding of direct mutagens. In some instances addition of liver S-9 increases the mutagenicity of DEPE, indicating the presence of compounds that act as promutagens. Pederson & Siak (30) found they could optimize culture conditions to observe mutagens requiring activation.

Lewtas (31) suggested using "revertants per mile" to compare mutagenic emission rates for vehicles. This approach led to the finding that diesel vehicles emit 45–800 times more mutagenic activity per mile than gasoline catalyst vehicles (32). Clark (16) summarized data obtained in a single laboratory for a number of vehicles, fuels, environmental conditions, and driving cycles, and found a range of between 2.4×10^5 and 19×10^5 revertants per mile. These data indicate the general similarity of mutagenic

emission rates for different light-duty diesel vehicles. Schuetzle & Perez (26) have observed that as a class, heavy-duty diesels emit only slightly more solvent-extractable material than do light-duty engines.

In studies using nitroreductase-deficient bacteria, DEPE was less mutagenic than in customary tester strains such as TA-98 (33, 34). These results and chemical analyses provide strong evidence that reduced nitro-PAH compounds cause much of the mutagenicity observed in strain TA-98. Rosenkranz et al (35) and Mermelstein et al (36) reported the extraordinary responsiveness of some strains to the nitroarenes and noted both the utility and the need for caution in using nitroreductase-deficient strains to assay complex mixtures. Rosenkranz & Howard (37) reviewed the structural basis of the activity of nitrated PAH.

Mammalian cell mutagenicity assays can also be used to evaluate DEPE. Positive dose-related effects have been found using the L5178Y mouse lymphoma assay without adding liver S-9. The mutagenicity was variable when liver S-9 was added (38). Li (39) observed most cytotoxicity in Chinese hamster ovary (CHO) cells when the concentrations of sera in the media were lowest. Addition of sera, lung S-9, lung S-9 plus cofactors, liver S-9, or liver S-9 plus cofactors had a protective effect. Li & Royer (40) reported a small, but dose-dependent, mutagenic response in CHO cells that was enhanced with the addition of liver S-9. They also found a greater than additive effect on mutagenicity of benzo(a)pyrene [B(a)P] plus DEPE over that observed with either B(a)P or DEPE alone. A mutagenic response was also observed in CHO cells that had phagocytized DEP (41).

Thilly et al (42) reported positive mutagenic responses for DEPE evaluated in a human lymphoblast gene mutation assay. However, DEPE was mutagenic only when tested with metabolic activation. These authors calculated that a substantial portion of the observed mutagenicity could be caused by fluoranthene, 1-methylphenanthrene, and 9-methylphenanthrene. The cell-transforming capacity of DEPE was assayed in BALB/c 3T3 mouse embryo cells with variable results (43). The lack of a clear dose-response relationship may have been related to the solubility of the extracts in the test system.

Chromosomal damage has occurred in animals exposed to DEP or DEPE. Pereira (44) reported that exposure of Syrian hamsters to high levels of DEP for several months increased the frequency of sister-chromatid exchanges in primary cultures of lungs from exposed animals. Intratracheal instillation of either DEP or DEPE also caused more sister-chromatid exchanges. An increase in the sister-chromatid exchange frequency occurred in CHO cells exposed in vitro to DEPE (45). A clear dose-response relationship for induction of chromosome aberrations was noted in CHO cells exposed to a potent DEP extract.

Mouse-Skin Tumor Bioassay

Nesnow et al (46) reviewed the results of extensive tests measuring the tumorigenic and carcinogenic potentials of DEPE applied to the skin of SENCAR mice. These studies compared the mutagenicity of DEPE to that of gasoline engine exhaust, coke oven emissions, and roofing tar, and extended the early observations of Kotin et al (1) that DEPE caused skin tumors in Strain A mice. A spectrum of tumor-initiating capacities was observed for DEPE from different engines, ranging from an inactive sample from one heavy-duty engine to a highly active sample from one light-duty engine. Using a log probit model with correction for background, the samples were ranked: B(a)P > coke oven mains ≥ the most active DEPE samples = roofing tar. A nonlinear Poisson model with background correction for tumor multiplicity provided the following ranking: topside coke oven > most active DEPE ≥ roofing tar ≥ intermediate DEPE sample = gasoline exhaust sample. The roofing tar– and coke oven–derived materials were effective promoters, but the DEPE samples were not. Data from complete carcinogenesis experiments after one year did not show DEPE to be complete carcinogens. In contrast, the coke oven– and roofing tar–derived samples and B(a)P were effective, complete carcinogens.

Disposition of Inhaled Particles

McClellan et al (47) emphasized the importance of complementing the results of in vitro studies with data from studies of the intact animal. A first step in this direction is to determine where inhaled DEP and their constituents are deposited in the body. Wolff et al (48) determined the deposition of radiolabeled particles, similar in size to DEP, in dogs. For particles with a mass median diameter of 0.02 or 0.1 μm, they observed pulmonary deposition of 32% and 25%, respectively. These results are in general agreement with those of Chan & Lippmann (49), who observed that approximately 18% of inhaled 0.2-μm particles landed in the pulmonary region of human volunteers. For risk assessment purposes, one could consider that 25–35% of inhaled DEP will be deposited in the pulmonary region.

Data are not available on long-term retention of very small particles in humans. This lack is of concern, since relatively insoluble particles larger in size than DEP have a long residence time in the pulmonary region (50). In the absence of data on DEP in people, this article reviews the data obtained in laboratory animals, from which estimates of human retention can be made.

Chan et al (51) and Lee et al (52) reported that rats exposed briefly to ^{14}C-DEP clear some of the DEP within hours or days. A smaller fraction is cleared with a half-life of 60 to 80 days. Strom & Chan (53), using these data,

developed a model to predict the long-term retention of DEP in the chronically exposed rat. The model under-predicted the retention of DEP beyond 20 weeks of exposure when the exposure concentration exceeded 250 μg DEP/m^3 for 20 hr per day, 5.5 days per week. The lung burdens of DEP did not platcau as expected, but continued to increase as a function of exposure time and concentration. The authors interpreted the buildup of DEP as evidence of impaired clearance and sequestering of DEP in aggregates.

Wolff et al (54) reported similar findings. Rats exposed to 3500 or 7000 $\mu g/m^3$ of diesel soot particles for 7 hr per day, 5 days per wk for 2 years accumulated more particles than did rats exposed to 350 $\mu g/m^3$. When administered a radiolabeled test aerosol after 2 years of exposure to DE, the control and 350-$\mu g/m^3$ rats had long-term clearance half-lives of 80 days, whereas those of the rats receiving 3500 and 7000 $\mu g/m^3$ were extended to 280 and 260 days, respectively. In considering these results, the investigator should recognize that the rat normally clears particles faster than man (55). In the guinea pig, as a further example, most of the inhaled DEP are very tenaciously retained, with little clearance between 10 and 432 days (52). The impaired clearance in the rat actually results in retention half-lives approximating those normally seen in man. It is not known if chronic exposure of people to high levels of DEP would increase retention times of particulate material in man. Our present data indicate that inhaled DEP in people have a half-life of several hundred days or more.

Disposition of Organic Constituents

This article has focused on the retention of the carbonaceous core of DEP. Of equal interest is the fate of the organic constituents of the DEP in view of their chemical identity and the established biological activity of DEPE. In attempting to relate in vitro data to studies more relevant to the mammalian body, several investigators (56–59) extracted DEP with many biological fluids and found, in general, that the mutagenic activity of DEP was reduced. Studies with macrophages and DEP suggest that macrophages can decrease the mutagenic activity of DEP, an observation consistent with other studies demonstrating that macrophages can metabolize particle-associated PAH to nonmutagenic metabolites (60). These data suggest the macrophages and biological fluids have a protective effect that reduces the mutagenicity of DEP constituents.

Another approach to obtaining information on the interaction of DEP constituents with tissues is to study the fate of radiolabeled constituents of DEP such as B(a)P and nitropyrene. Sun et al (61) reviewed the literature on the disposition of inhaled particle–associated organic compounds. Sun et al (62) studied the retention of inhaled B(a)P adsorbed on the surface of DEP. There was an initial phase of rapid clearance from the respiratory tract,

followed by a second phase of slower clearance. A substantial portion of the B(a)P and its metabolites were retained, with a long-term clearance half-life of 18 days. These investigators demonstrated that up to 20 days after inhalation, approximately two-thirds of the retained radiolabel was B(a)P, with the remainder identified as phenol and quinone metabolites. These results are similar to those previously reported by Henry and coworkers (63), who observed that following intratracheal instillation into Syrian hamster lungs, B(a)P coated on carbon particles was retained much longer in the lung than when it was coated on aluminum or ferric oxide particles or given as a pure compound. Different types of carrier particles, including carbon black, effectively enhance respiratory tract carcinogenesis by B(a)P (64).

The fate of 1-nitropyrene inhaled as a coating on DEP or as a homogeneous ultrafine aerosol of the pure compound has been studied (65, 66). The 1-nitropyrene associated with particles, and especially with DEP, enhanced the long-term retention of 1-nitropyrene in the respiratory tract. Substantially more nitropyrene was found in the liver and kidneys when the nitropyrenes were inhaled with DEP.

Effects of Chronic Exposure

RESPIRATORY TRACT CANCER One of the ultimate concerns for exposure to DE is that it may result in lung cancer. The positive mutagenicity results in several assays and tumorigenicity in the mouse-skin assay with DEPE indicate the potential carcinogenicity of inhaled DEP. However, the positive results in these assays have generally been obtained under extraordinary conditions, i.e. with high concentrations of extracts obtained by treating DEP with strong organic solvents. This approach presents the biological system with a large quantity of test material in a very short period of time and bypasses many of the protective mechanisms encountered by inhaled DEP. To directly assay for carcinogenicity, Syrian hamsters, mice, and rats have been exposed to various dilutions of whole DE (11). In several studies, animals were also exposed to exhaust from which the particles had been removed, whereas in other studies, animals were administered known carcinogens and exposed to DE.

Studies with Syrian hamsters exposed to whole DE or particle-free DE have shown no tumor production (67–69). Recognizing the difficulty of detecting a potential small increase in neoplasia, Heinrich (68) pretreated some of the hamsters with a known carcinogen, diethylnitrosamine (DEN), and then exposed them to DE. Exposure to whole or particle-free DE did not produce respiratory tract tumors. With a high dose of DEN (4.5 mg/kg), a 45% baseline incidence of papillomas of larynx and trachea was observed, which increased to 66% and 70% with inhalation of particle-free and whole DE, respectively. The similarity of the response in both groups suggests that it may have been promoted by irritant gases in the exhaust. Takemoto et al (70)

observed a greater than additive effect on lung tumor induction in F344 rats exposed to DE and treated with a known carcinogen, di-isopropanol-nitrosamine (DIPN), compared to those exposed only to DIPN or DE.

Studies with mice exposed to DE have given variable results. Kaplan et al (71) and Orthoefer et al (72) exposed Strain A mice, which normally have a high spontaneous incidence of lung adenomas, to DE. The lung tumor incidence in the DE-exposed mice was not increased above that of controls. Indeed, Orthoefer et al (72) reported a decreased incidence in the DE-exposed mice. Stoeber (69) exposed female NMRI mice to DE at a concentration of 4.0 mg/m^3 for up to 120 weeks (19 hr per day, 5 days per week), and found the incidence of adenocarcinomas in both whole-exhaust and particle-free exposed groups was significantly higher than that in controls. The incidence of adenomas was not affected. Takemoto et al (70) exposed C57BL/6N mice to DE at 2–4 mg/m^3 for 4 hr per day, 4 days per week, and observed an increased incidence of both adenomas and adenocarcinomas at 19–28 months.

In contrast to the negative or variable results observed in Syrian hamsters and mice, five laboratories have found a statistically significant increase in lung tumor incidence in rats chronically exposed to DE. Figure 1 is a compilation of the results of six major studies (69, 73–77) in which rats were exposed to DE. To aid in comparing the results of the several studies, the exposure conditions have been normalized and expressed as mg · hr/m^3 · week. For example, an exposure of 7.0 mg/m^3 for 7 hr per day, 5 days per week would be recorded as a 245 mg · hr/m^3 · week exposure. The studies used F344 rats, except for the study reported by Stoeber (69), which used Wistar rats. The investigators exposed the rats to DE for 24–30 months and typically observed them for 30 months. The exception was the study reported by White et al (73), which involved only 15 months of exposure and 8 months of recovery. The studies varied in other experimental details, i.e. engine type and operating conditions, fuel, and exposure duration. Nonetheless, the similarity of the results is striking. Lung tumor incidence clearly increased with exposures at high levels ($>$ 100 mg · hr/m^3 · week) for 2 years or more.

The tumors were observed late in the studies. For example, Mauderly et al (75) identified 81% of their tumors after 24 months of exposure. The late occurrence of the tumors may account for Lewis et al (78) not observing an increase in lung tumors in rats exposed for 2 years to DE alone or in combination with coal dust. Likewise, this delayed incidence may explain the lack of a statistically significant increase in lung tumor incidence in the study reported by White et al (73).

Four types of tumors have been observed, all of which appear to have derived from epithelial cells (75–79). Bronchoalveolar adenomas were typically small in size, were composed of cuboidal cells resembling Type II or Clara epithelial cells, and were not invasive. The adenocarcinomas observed

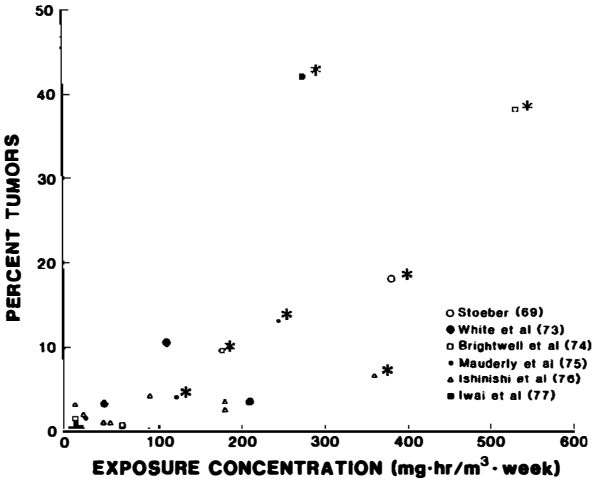

Figure 1 Lung tumor incidence in rats related to normalized exposure concentration of DE. Values marked with * differed from controls by statistical test.

were similar to the adenomas, but had central areas with hyperchromatic cells of nonuniform size arranged in irregular patterns. There were increased numbers of mitoses and invasion of adjacent vessels or interstitial connective tissue. Squamous cysts, composed of large keratin cores surrounded by well-differentiated uniform squamous epithelium, were frequently observed. Their malignant counterpart, squamous cell carcinomas, were similarly observed. The cells at the periphery of these carcinomas were less differentiated and the tumors were locally invasive. Metastasis to pulmonary lymph nodes or other organs was rare. The classification of the squamous lesions has been discussed by Mohr et al (79).

The pathogenesis of the tumors is not known at this time. It is tempting to speculate, based on the known mutagenicity of DEPE, that the tumors result from the organic compounds associated with the inhaled DEP. With rats from the Mauderly et al (75) study, Wong et al (80) observed increased numbers of adducts in DNA extracted from lungs of DE-exposed, as compared to control, rats. This observation lends support to a genetic mechanism for induction of the tumors.

Alternatively, Vostal (81) has suggested that the lung tumors in rats exposed to high levels of DE may be due to the overloading of the normal clearance mechanisms, accumulation of DEP, and nonmutational, epigenetic mechanisms of cancer induction. He further suggests a threshold relationship between administered or retained dose of DEP and lung tumor incidence. He notes that increased incidences of lung tumors also occur in rats exposed to high levels of shale dust, solvent-refined coal solids, titanium dioxide, tita-

nium tetrachloride, and coal dusts, which are generally viewed as "nuisance" dusts.

At this time it is not possible to determine the relative role of genetic vs epignetic mechanisms in the initiation or promotion of lung tumors in the DE-exposed rats. Quite possibly, both mechanisms are involved in some complex interactive manner. Additional research should help clarify the situation. For example, it would be useful to assess the carcinogenicity of carbon black particles devoid of organic compounds and with various added amounts of selected organic compounds typically present in DEP. The rate of formation and repair of DNA adducts in DE-exposed animals should be determined both during the exposure period and following cessation of exposure. Finally, lung tumors in diesel-exposed animals should be evaluated for the presence of oncogenes.

NONCANCER ENDPOINTS Many of the chronic exposure studies were designed to detect noncarcinogenic functional disorders as well as to assess the carcinogenicity of DE. The specific studies evaluated the deposition and clearance of DEP (as already noted), the pathological changes in lungs and associated lymph nodes, pulmonary function, clearance of particles, susceptibility to infectious agents, biochemical changes, and immunological alterations.

The pathological changes observed in the respiratory tracts of several laboratory animal species exposed to DE have been described (10, 59, 82). The major gross finding is an exposure concentration–related dark discoloration of the lungs and thoracic lymph nodes. Histologically, the number and size of alveolar macrophages increase. DEP are found in macrophages in alveoli, alveolar interstititium, peribronchial and perivascular interstititium, and lymphatic channels, in histocytes in the sinusoids of thoracic lymph nodes, in Type 1 epithelial cells, and in eosinphils. The Type 2 pneumocytes are increased in number and size in the alveoli that contain DEP-laden macrophages. At the highest levels, numbers of polymorphonuclear leukocytes also increase. The tissue response to DEP varies from no detectable changes to partial or complete obliteration of alveoli and replacement of them with fibrous connective tissue, which sometimes incorporates aggregates of DEP. There were foci of metaplastic epithelium in alveoli and terminal bronchioles.

Mauderly et al (83) reported a restrictive disorder of pulmonary function at the high levels of exposure to DE. This disorder included increased stiffness of the lung parenchyma and lower lung volumes, with reductions in total lung capacity and vital capacity per kg body weight. Intrapulmonary gas was distributed less uniformly, and alveolar gas exchange was impaired. Gross (84) reported similar trends in rats exposed to moderate levels of DE,

although the changes were not statistically significant through 612 days of exposure. Pepelko (85) reported similar statistically significant functional changes in rats and Chinese hamsters exposed to high levels of DE for 6 months.

Campbell et al (86) observed that DE-exposed mice were more susceptible to experimentally induced infection with *Streptococcus pyogenes*. They suggested that this enhanced susceptibility may be caused by the NO_2 and acrolein vapor in the DE. The DE exposure did not enhance the mortality in response to influenza virus A/PR8-34 or *Salmonella typhimurium*.

Bice et al (87) reported exposure to DE affected pulmonary immunological function. They observed exposure concentration– and exposure time–related increases in the total number of cells in thoracic lymph nodes of rats and mice. These authors also noticed a trend toward increased numbers of IgM antibody–forming cells per 10^6 lymphoid cells after intratracheal instillation of particulate antigen in rats.

Henderson et al (88) reported alterations in the biochemical and cellular constituents recovered by lavage from airways of DE-exposed rats. The changes were consistent with an inflammatory response after 6 months of exposure to either 3.5 or 7.0 mg/m^3, but not with 0.35 mg/m^3, of DE. The changes persisted throughout the 30-month study.

EPIDEMIOLOGICAL STUDIES

A number of epidemiological studies have been conducted on DE-exposed populations. Although diesel engines have been used for many decades, their emissions are rapidly diluted, and relatively few individuals have been exposed to high concentrations of DE. Thus, populations with sufficient exposure and of appropriate size to warrant study are difficult to identify. In addition, because respiratory disease is of particular interest, the complications of exposure to other inhaled pollutants, and especially cigarette smoke, are important confounding variables (89). If the disease, or diseases, resulting from exposure to DE were unique, which they are not, the task would be much easier.

Four major populations exposed to DE have been studied: transportation workers, operators of heavy construction equipment, railroad workers, and miners. The most thoroughly evaluated set of data is that collected on the London Transit Authority Workers (90, 91). The overall annual lung cancer rate for the London Transit Authority Workers exposed to DE was 159 per 10^5, which was significantly lower than the corresponding rate of 202 per 10^5 for males in Greater London during the period of the study, 1950–1974. Harris (92, 93) analyzed these data using a linear exposure-effect relative risk model. This model assumes that the increased risk from exposure to DE is

proportional to the average lifetime exposure to the concentration of DE in the air multiplied by the worker's normal risk. In his first analysis (92), he placed a quantitative limit on the probability that a carcinogenic risk had gone undetected. With this approach, he calculated the 95% upper confidence limit to be on the order of a 5×10^{-4} proportional increase in lung cancer incidence per unit of cumulative lifetime exposure, where one unit of exposure is equivalent to inhaling a concentration of 1 μg DEP/m^3 for one year. In his second analysis, Harris (93) compared the ratios of the observed cases to age-standardized expected cases for three job-grouping subpopulations. The observed-to-expected ratios for lung cancer for the three job groupings were ordered in relation to the degree of presumed exposure to DE. This order allowed the estimation of the maximum lung cancer risk to be 1.23×10^{-4} per unit of cumulative exposure. The original data set failed to show an effect of DE exposure on lung cancer incidence; however, the calculations serve to indicate the magnitude of a potential effect.

Wong et al (94) studied members of a heavy-construction-equipment operators union to evaluate the mortality of 34,156 male members between January 1, 1964 and December 31, 1978 for comparison with that of all white males in the United States. They observed that the standardized mortality ratio (SMR) for all causes of death was 81, which was significantly lower than the comparison population. The SMR for lung cancer was 99, with risk increasing with the interval between first exposure and death. The SMR for lung cancer was significantly increased for retirees; however, there was no association demonstrated between DE and lung cancer. Indeed, the SMR for lung cancer was lower for occupations believed to result in high DE exposures.

Hall & Wynder (95) reported a case-control study of lung cancer in which DE exposure was considered. They observed a strong association of lung cancer with cigarette smoking and a twofold increase in lung cancer for those exposed to DE when cigarette smoking was eliminated as a factor. When allowance was made for smoking, the difference for the DE-exposed cases was eliminated.

There have been three reports of case-control studies of bladder cancer that have considered DE exposure. These studies appear logical, considering the potential for urinary excretion of organic constituents of inhaled DE. Silverman et al (96) noted an increased risk of lower urinary tract cancer in truck drivers, with risk increasing with duration of employment. The relative risk for operators of diesel trucks was 11.9 times that of people who did not drive trucks. Some allowance was made for smoking in nontruck drivers, but not for the diesel drivers. In a second report, Silverman et al (97) noted truck drivers or delivery men had a 50% increase in risk of bladder cancer. Higher risks were also reported for bus and taxi cab drivers. Hoar & Hoover (98)

reported a 50% increased risk of bladder cancers in truck drivers, which was inconsistently associated with duration of driving and was higher in drivers reported to have been exposed to DE. Hoar & Hoover (98) reported taking allowance for cigarette smoking and coffee drinking. Wynder et al (99) found an increased risk of bladder cancer in individuals having high exposure to DE in the absence of allowance for cigarette smoking, which was independently shown to result in an excess risk of bladder cancer. When allowance was made for smoking in the DE-exposed cases, the excess of lung cancer was eliminated.

The importance of considering multiple confounding factors in interpreting cancer epidemiology studies has been emphasized by Wynder & Higgins (89). They documented the unusual cigarette smoking and dietary fat intake of truck drivers. They are not convinced that the excess risk of bladder cancer reported by others is due to DE exposure or, indeed, even to occupation.

Kaplan (100), studying the cause of death of 6,506 US railroad workers, found no association between exposures to DE and lung cancer deaths. However, only 154 cases of lung cancer were recorded in the population, and diesel locomotives did not exceed 50% of all Class I locomotives in the United States until 1952 (101). Thus, Kaplan's population had been exposed to DE for a relatively short time. Howe et al (102) reported on a cohort study of 43,826 male pensioners of the Canadian National Railway Company. They observed highly significant exposure-response relationships for elevated risk of lung cancer in individuals employed in occupations involving exposure to DE and coal dust. The relative risk of those possibly exposed or probably exposed to DE were 1.20 and 1.35, respectively. Almost identical values were observed for coal dust exposures. The authors were not able to determine the role of the possible confounding effects of coal dust and asbestos exposure and of smoking. The study did not report quantitative information on duration or levels of exposure to DE. Such information is essential for deriving quantitative risk estimates for exposure to DE.

Schenker & Speizer (101) are making a large retrospective cohort study of approximately 80,000 railroad workers in the United States. In addition, they are conducting a case-control study of 300 incident lung cancer cases and matched controls in railroad workers and monitoring the environment in an attempt to quantitate exposures to DE. In a preliminary report Schenker et al (103) noted the SMR for the railroad workers compared to the US national rates was 87 for all causes of death and 85 for lung cancer. The relative risk of those exposed to DE compared to those not exposed was 1.42 ± 0.50. The low and high risk of lung cancer for DE exposure was 1.50 and 2.77, respectively. These data are suggestive of a DE exposure effect, however, they were not corrected for cigarette smoking. Garschick et al (104), studying

the same population, has reported an exposure time-related increase in the relative risk for lung cancer among workers exposed to DE as compared to cohorts with little exposure.

Waxweiler et al (105) studied potash workers and found no significant mortality differences between DE-exposed and nonexposed miners. The population size was not large, and the 24 years of maximum diesel exposure was not great. Wheeler et al (106) reported on exposure data obtained in dieselized coal mines as part of a five-year effort initiated by the US National Institute of Occupational Safety and Health to study health implications of diesel engine use in coal mines.

ESTIMATING RISK OF LUNG CANCER

A major objective of the research on DE emissions has been to establish quantitative estimates of the potential health risks of exposure of people to DE. Three approaches (Table 1) have been taken based on (*a*) epidemiological observations of persons exposed to DE, (*b*) comparative potency studies, and (*c*) observations of lung tumors in rats. The approach using epidemiological data was reviewed earlier (107). The comparative potency approach uses information from epidemiological studies of known human respiratory tract carcinogens (coke oven emissions, roofing tar, and cigarette smoke) in combination with bioassay data on these same materials and DEPE (27, 108, 109). This approach assumes that the materials have the same basic mechanisms of action. The third approach, which uses data on lung tumor induction in rats, assumes that the mechanisms by which the lung tumors were produced in rats exposed to high levels of DE are operative in people exposed to low levels of DE. The value reported (109) was derived using the rat tumor data of Mauderly et al (75). To facilitate comparisons among the risk estimators (Table 1), these studies have all been applied to a common base, namely a population of 230×10^6 persons with an average life span of 70 years, annual deaths from lung cancer of 1×10^5, and continuous exposure to 1 μg of DEP/m^3.

The 1 μg/m^3 level of exposure approximates the average calculated to occur if 20% of the light-duty vehicles in the United States in 1995 were diesel powered and emitted 0.12 g DEP/km (107). In aggregate, this emission would represent 60,000 metric tons of DEP per year. Although the average exposure would approach 1 μg/m^3 in the projected scenario, individual exposures would vary greatly, e.g. parking garages would contain a DE concentration of 80 μg/m^3; typical metropolitan street canyons, 10 μg/m^3; cities, 0.5 μg/m^3; and rural areas, 0.05 μg/m^3. It now appears very unlikely that the use of light-duty diesel vehicles will even approach the 20% level in the United States. Nonetheless, the approach used is illustrative of the use of

Table 1 Comparison of lung cancer risk estimates for DE exposure[a]

Approach	Reference	Risk model	Risk estimator	Lung cancer (cases/yr)
Epidemiological data on diesel exhaust exposure	(92)	Proportional	$\frac{5 \times 10^{-4} \text{ increased lifetime risk}}{\mu g/m^3 \text{ for 1 yr}}$	3500
Epidemiological data on diesel exhaust exposure	(93)	Proportional	$\frac{1.23 \times 10^{-4} \text{ increased lifetime risk}}{\mu g/m^3 \text{ for 1 yr}}$	860
Comparative Potency	(93)	Proportional	$\frac{0.35 \times 10^{-4} \text{ increased lifetime risk}}{\mu g/m^3 \text{ for 1 yr}}$	245
Comparative Potency	(107)	Absolute	$\frac{0.3 \times 10^{-4} \text{ lifetime risk}}{\mu g/m^3}$	100
Comparative Potency	(108)	Absolute	$\frac{0.1 \text{ case/yr}/10^5 \text{ person}}{\mu g/m^3}$	230
Rat lung tumor data	(109)	Absolute	$\frac{0.12 \times 10^{-4} \text{ lifetime risk}}{\mu g/m^3}$	40

[a]See text for underlying assumptions.

risk assessment methodology combining quantitative risk estimators with estimates of exposure to assess risk to a population (9, 107).

In considering the calculated risks, it is appropriate to keep several points in mind. First, the range of the risk estimates is indicative of our current lack of certainty regarding human carcinogenic risks of DE exposure. Second, this range includes recognition that our current level of knowledge does not exclude the possibility that no cancers are attributable to low-level exposures to DE. Third, several of the estimates are based on proportional risk models. These models assume that the added risks occur primarily in the population with the highest base incidence, i.e. cigarette smokers. Fourth, to place the potential risks in perspective, it is currently estimated that in the United States approximately 100,000 lung cancer deaths occur per year in smokers and an additional 10,000 lung cancer deaths occur per year in nonsmokers.

SUMMARY

Diesel-powered vehicles emit substantially more particles than do gasoline-powered vehicles with contemporary emission control systems. The DEP are submicron in size and readily inhaled. Approximately one-fourth of the particle mass inhaled by people is deposited in the pulmonary region, some of which is retained with a half-life of several hundred days. In animal studies, exposure to high levels of DEP overwhelms the normal clearance mechanisms and results in lung burdens of DEP that exceed those predicted from observations at lower exposure concentrations.

A variable amount of the mass of DEP is extractable with strong organic solvents. The extracted material contains more than a thousand individual compounds and is mutagenic in a number of bacterial and mammalian cell assays. Bioassay-directed chemical analysis of DEP has identified several hundred compounds. Many are PAHs, some of which are considered to have human carcinogenic potential. A number of nitrated compounds have been identified that account for a significant portion of the mutagenicity assayed in bacteria. The mutagenicity of the DEPE is generally reduced by addition of an S-9 cellular fraction or of serum proteins. Macrophages rapidly reduce the recoverable mutagenic activity associated with DEP. These findings support a hypothesis that detoxification of DEP-associated organics occurs rapidly in vivo. The association of benzo(a)pyrene and nitropyrene with DEP prolongs their retention in the lungs. This increased retention suggests the need to clarify the relative importance of competing mechanisms that detoxify particle-associated compounds and those that serve to enhance the retention of toxicologically important compounds.

Some extracts of DEP evoke tumorigenic responses in skin-tumor bioassays, suggesting their carcinogenic potential in mammals. A number of

large-scale studies have been conducted with laboratory rodents to evaluate the effects of chronic inhalation exposure to DE. An increased incidence of lung tumors, some of which were diagnosed as malignant, was observed in 5 studies with rats following exposure for 2 or more years to high levels of DE. Most of the lung tumors were observed after 2 years. Similar studies in Syrian hamsters have yielded negative results. Studies with mice have given mixed results. The results of some studies with laboratory animals exposed to DE and known carcinogens suggest that exposure to DE enhances the effect of the known carcinogens. The specific mechanisms of tumor induction in the DE-exposed rats are unknown. Hypotheses and experimental data have been advanced in support of both genetic and epigenetic mechanisms of action of the DE. Clarification of this issue has important implications in considering the use of the rat data for estimating the risk to people of low-level exposure to DE.

A number of epidemiological studies have been conducted to evaluate the risk of lung or bladder cancer in people exposed to DE. Analyzing these studies has been made difficult by the relatively low DE exposure of people and the substantial impact of confounding factors such as cigarette smoking. Taken in aggregate, the epidemiological evidence for DE exposure inducing lung or bladder cancer is negative or, at most, only suggestive of an effect.

The risk of lung cancer in humans from chronic exposure to DE has been estimated using (*a*) epidemiological data, (*b*) a comparative potency approach, and (*c*) tumor data from rats chronically exposed to DE. Applying the various risk estimators to the US population of 230×10^6 persons, and assuming exposure to 1 μg of DEP/m^3 of air for 70 year yields, estimates 40, 100, 230, 245, 860, or 3500 lung cancers per year in excess of the more than 10^5 lung cancer cases per year expected from other causes, principally smoking.

If 20% of the light-duty vehicles in the United States were to be diesel powered and to emit 0.12 g/km, total DEP emissions would be 60,000 metric tons per year. This amount would result in average rural exposures of 0.05 μg/m^3, urban exposures of 0.5 μg/m^3, and some situations with exposure of > 5 μg/m^3. The corresponding excess lung cancer risk is estimated to be fewer than 200 deaths per year; however, our current knowledge does not exclude the possibility that no lung cancer deaths could be attributed to low-level DE exposure.

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