TOXICOLOGY OF DIESEL ENGINE EMISSIONS

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INTRODUCTION

Diesel engine exhaust is a complex mixture of gases, vapors, and particles (soot), each consisting of a large number of compounds (1). Concern for health risks from diesel exhaust has focused primarily on the potential lung carcinogenicity of inhaled diesel soot, because of its respirability and its content of mutagenic organic compounds. Research on the potential lung cancer risk from inhaled diesel exhaust has taken three forms (2). First, researchers at tempted to identify the compounds responsible for its mutagenicity in cultured bacteria and mammalian cells, and to estimate cancer risk by comparing the mutagenicity of diesel exhaust to those of known human lung carcinogens. Second, several long-term inhalation studies in rats, mice and Syrian hamsters were conducted in the U.S., Europe, and Japan, followed by additional studies to determine the interpretive value of the results from rats. Third, epidemiological studies of occupational populations thought to have high diesel exhaust exposure were conducted. This paper reviews laboratory studies of the mutagenicity and carcinogenicity of diesel exhaust, and sum-marizes our present interpretation of the re-sults.

GENOTOXICITY OF DIESEL SOOT

Recognition of the potential carcinogenicity of diesel soot occurred in 1955, when Kotin et al. demonstrated that solvent extracts of soot caused cancer when applied to mouse skin (3). Concern was elevated in the late 1970s when U.S. Environmental Protection Agency researchers reported that soot extract was a direct-acting mutagen in bacteria (4), concurrently with a predicted increase of diesel engines among the U.S. light-duty fleet. The majority of the mutagenic activity in bacteria and mammalian cells was subsequently found to be attributable to polycyclic aromatic hydrocarbon (PAH) compounds, and especially the nitro-PAHs (5).

The extent to which the soot-associated organic mutagens are released after deposition in the lung remains unclear (2,6). Because the aqueous lung environment is quite different from the strong solvents, heat, and ultrasonic energy typical of laboratory extractions, it is extremely unlikely that the entire organic fraction would be released *in vivo*. Extractions using lung fluid simulants remove little mutagenic activity; however, some mutagenic activity is released from soot by lung macrophages (mobile scavenger cells) in culture. Mutagenic activity has been identified in the urine of rodents exposed to diesel soot by the intragastric, intraperitoneal, and subcutaneous routes, but no urinary mutagenic activity has been observed following exposure of rodents to soot by inhalation. Increases in the levels of DNA adducts (foreign molecules attached to DNA) have been detected in lungs of rats exposed by inhalation to high concentrations of diesel exhaust (6), but this finding is difficult to interpret. Similar increases in adducts have been observed in parallel groups exposed to carbon black and other particles having very little mutagenic activity (6-8). In addition, the increases occur primarily in adducts that also occur in unexposed rats (7). Finally, the increases in lung DNA adducts do not appear to progress during long-term exposures (7).

CARCINOGENICITY IN ANIMALS EXPOSED TO DIESEL EXHAUST BY INHALATION

By the mid-1980s, several long-term studies of rodents exposed by inhalation to whole, diluted diesel exhaust had been conducted. These initial studies included 11 studies of rats

conducted in nine laboratories, five studies of Syrian hamsters conducted in four laboratories, and five studies of mice conducted in five laboratories (2,9). Although the studies used different strains of animals, engines and operating conditions, fuels, exposure concentrations, weekly exposure patterns, and total lengths of exposure, a consistent pattern of results emerged.

Whole diesel exhaust, inhaled repeatedly at high concentrations for 24 mo or longer, caused increased incidences of lung tumors in rats. The tumors were located in peripheral lung tissue, were of epithelial (air space lining cell) origin, and consisted of both benign and malignant types. Another lesion, keratin cysts, also occurred at the higher exposure levels and were reported as benign tumors by some groups; however, these cysts are not currently considered "tumors" in the U.S. The tumor response did not appear to be influenced strongly by the strain of rat or by engine type, fuel type, or engine operating condition, but the response was consistently greater in females than in males.

The results of the most comparable and robust studies, all involving exposures of substantial numbers of rats for 30 months or longer, are presented graphically in Figure 1. The tumor response clearly demonstrated an exposure-response relationship, and was approximately proportional in a nonlinear manner to the exposure rate expressed as the weekly soot concen-tration x time product. The lowest exposure rate causing a significant increase in lung tu-mors in rats exposed for 30 months or longer was approximately 122.5 $mg \cdot h \cdot m^{-3}$, or 3,500 $\mu g/m^3$ x 7 h/d x 5 d/wk. Lower exposure rates did not cause significant carcinogenicity. Tu-mors were observed late in the life span, most near or after 24 months of exposure. Studies containing no groups with significant increases either involved only exposure rates below 120 $mg \cdot h \cdot m^{-3}$ or

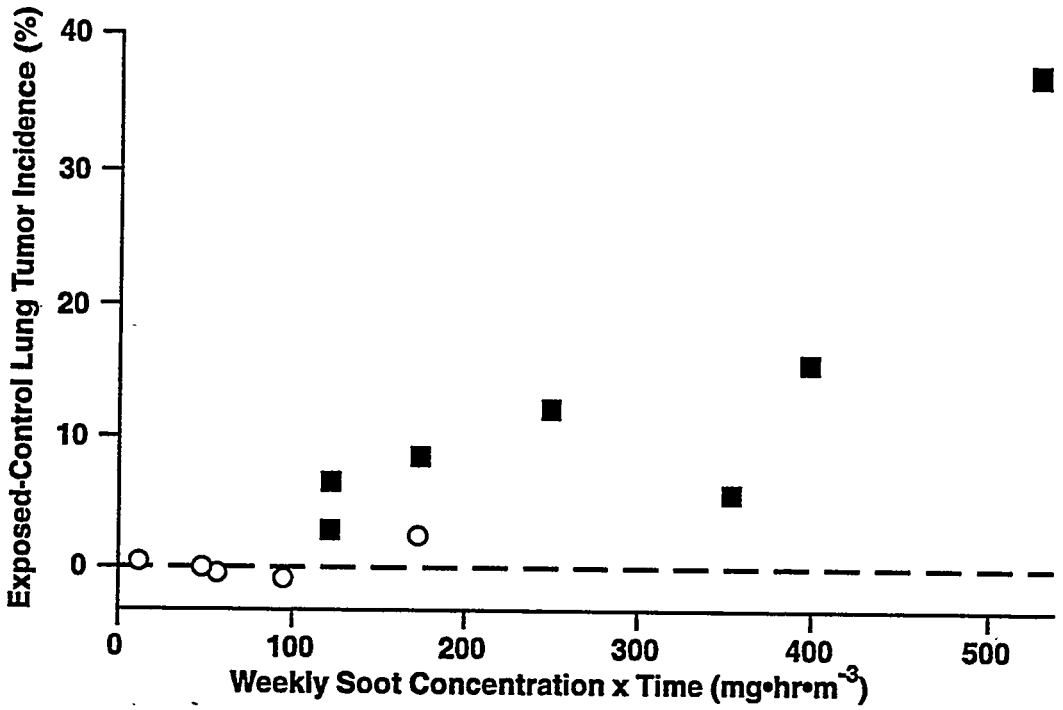

Figure 1. Relationship between diesel exhaust exposure and lung tumor response in rats exposed for a minimum of 30 months. Lung tumor response is expressed as the net (exposed -control) incidence, with the dashed line indicating zero net response. Exposure is expressed as the weekly concentration x time product. Filled squares represent groups with stati-stically significant increases in lung tumor incidence. Open circles represent exposed groups without significant increases in lung tumor incidence. Reproduced from reference (2).

exposures and observations of rats for less than 24 months. There was no evi-dence of a carcinogenic effect in other organs of rats. Exhaust filtered to remove soot did not cause tumors.

Exposures causing lung tumors in rats resulted in deposition of soot in the lung at rates exceeding the capacity of normal particle clearance, causing a slowing of clearance and a progressively increasing lung burden of soot (10). Exposures causing tumors also caused a progressive pneumoconiosis consisting of inflammation, fibrosis, hyperplasia (increased cell numbers) of alveolar and terminal bronchiolar epithelium, and epithelial metaplasia (altered cell types) (11). Conversely, exposures that did not cause substantial build-up of soot in the lung and progressive pneumoconiosis did not increase the incidence of lung tumors.

Parallel studies of mice and rats produced mixed results. Increased incidences of lung tumors were produced in a few groups of mice of strains that are genetically sensitive to chemical carcinogenesis, but results were equivocal or negative in ordinary strains (2,12). Soot accumulated in lungs of mice in amounts equivalent on a size basis to the accumulations in rats, and mice also developed chronic inflammation and fibrosis (13); however, mice had little epithelial hyperplasia and metaplasia.

Parallel studies of rats and Syrian hamsters demonstrated that soot exposure rates causing lung tumors in rats did not increase the lung tumor incidence in hamsters. Soot accumulated in the hamster lungs and caused lung pathology, but no increases in lung tumors were reported.

INTERPRETATION OF CARCINOGENICITY RESULTS FROM ANIMALS

By the late 1980s, data accumulated suggesting that lungs of rats respond similarly to heavy loading with diverse types of poorly soluble, nonfibrous particles (14). Several particles, including some with no organic mutagens, caused a similar syndrome of inflammation, fibrosis, epithelial hyperplasia and metaplasia, and epithelial tumors of the same type induced by diesel soot. Carcinogenesis by particles with no organic mutagens raised doubts that the diesel soot-associated organic mutagens were responsible for the pulmonary carcino-genicity of diesel exhaust in rats. The impor-tance of the soot-associated organic mutagens in the rat lung tumor response to diesel soot was tested in two studies. Nikula et al. (15) exposed rats 6 h/d, 5 d/wk for 2 y to either diesel exhaust at 2.5 or 6.5 mg/m^3 soot or identical concentrations of similar-sized carbon black having negligible mutagenic content. The diesel soot and carbon black exposures caused essentially identical lung tumor re-sponses, despite the large difference in muta-genicity of the two materials. Heinrich et al. (16) exposed rats to multiple concentrations of diesel soot, to mutagen-poor carbon black or to titanium dioxide, and found that the three materials had equal carcinogenic potency. These results demonstrated that the soot-associated organic mutagens, which were the principal concern for potential human cancer risk, are not important in causing the lung tumors in rats exposed to diesel exhaust.

The difference between lung tumor responses of rats and other rodents is not unique to diesel soot (17). Ten particles of different types have been shown to cause lung tumors in rats, but not mice, exposed by inhalation. Six of these materials have also been shown to be negative in hamsters, and four have not been tested in that species. In addition, beryllium metal and nickel oxide, like diesel soot, are lung carcin-ogens in rats exposed by inhalation, but only produce equivocal results in mice. The difference between rats and mice is not consistent for all particulate materials however; cadmium oxide, calcium chromate, coal tar aerosol, and cobalt sulfate cause lung tumors in both rats and mice exposed by inhalation.

Little is known about the long-term responses of non-rodent animal species to chronic, heavy particle exposure. Snipes (18) reviewed the scanty information available for non-rodent species and drew the tentative conclusions that particles tend to accumulate differently in

rats and non-rodent species and that the cellular responses of non-rodent species to deposited particles are typically less than that of rats. Nikula et al. (19) recently provided support for this conclusion by demonstrating that diesel soot and respirable coal dust accumulate in different preferential locations in lungs of rats and non-human primates exposed in parallel by inhalation. These materials accumulated primarily in alveoli in rats, but largely in the interstitium in monkeys. Also, in contrast to rats, monkeys had little epithelial proliferative re-sponse to either material.

CONCLUSIONS

There is a large amount of laboratory data on the toxicology of diesel emissions. Laboratory studies have shown clearly that diesel emissions contain toxic materials, but laboratory data do not allow confident prediction of the existence, nature, or magnitude of the potential human health risks. Attention has been focus-ed largely on lung cancer risk. The present laboratory evidence indicates that the lung tumor response of rats to heavy, chronic exposures to diesel soot should not be used to develop quantitative estimates of lung cancer risk in humans exposed to environmental levels. The tumor response of rats occurs only at high exposure concentrations, and has an apparent exposure threshold. The tumor response of rats can not be extrapolated to other rodents. Monkeys do not develop the epithelial proliferative response to diesel soot that occurs in rats. Moreover, there is no evidence that the lung epithelial cell proliferation and tumor development observed in rats is characteristic of the response of human lungs to heavy particle loading.

The inapplicability of the rat results to quantitative estimates of human lung cancer risk does not prove that diesel exhaust presents no risk to humans. The toxicological results give no strong basis for excluding diesel exhaust when considering the total exposure of humans to inhaled mutagens in the workplace or general environment. On the other hand, our present knowledge of the toxicology of diesel exhaust does not provide a basis for assigning any unique human lung cancer risk to diesel exhaust other than that presented by any material containing similar types of mutagenic activity.

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