Chapter 8
Potential Health Effects of Exposure to Diesel Engine Exhausts

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Abstract

Diesel exhaust (DE) is a complex mixture of particulate matter, condensible vapors, and gases, with a composition that can vary depending upon engine type, fuel type, and operating conditions. Elevated exposure levels of DE have been linked with both cancer and non-cancer health risks, primarily in studies of exhaust from diesel technology typical of the engines, fuels, and lubricants in use prior to 1988 when diesel-engine emissions were not regulated in the United States. Prompted by increasingly stringent emission standards implemented by the US Environmental Protection Agency over the last two decades, major advances have occurred in diesel technology that have resulted in quantitative and qualitative changes in diesel-engine emissions, including dramatic reductions in emitted diesel exhaust particulate (DEP), nitrogen oxides (NO\textsubscript{x}), and other components.


Diesel engines differ from spark-ignition, gasoline engines in that the fuel-air mixture is ignited by the heat of compression. Diesel engines combust hydrocarbon fuels with oxygen to produce mechanical energy, and thus, at the fundamental level, the products of combustion found in "diesel exhaust" (DE) are gaseous carbon dioxide (CO\textsubscript{2}) and water (H\textsubscript{2}O). Because combustion is generally incomplete, and because diesel fuel contains some level of impurities, many other chemical compounds are present in DE, including a small quantity of airborne particulate matter (PM), called "diesel exhaust particulate matter" (DEP).
At the outset, it should be pointed out that diesel-engine exhaust, its composition, and its potential health effects have been the subject of frequent review. Among the major reviews is the 2002 US EPA review entitled "Health Assessment Document for Diesel Exhaust," a 671-page analysis (1). Additional, contemporary reviews include Hesterberg et al. 2006, 2009a, 2009b, 2010; Gamble, 2010; Institute of Medicine, 2005; Mauderly and Garshick, 2009; Pronk et al. 2009; and Wichmann, 2006 (2-10). Older major reviews include the 1989 International Agency for Research on Cancer (IARC) monograph (11), the 1995 report by the Health Effects Institute (12), and the health-assessment documents developed by the California EPA (13).

In practice, DE is a complex mixture of particulate aerosol – DEP, condensible vapors, and gases. DE composition varies widely depending upon engine type, fuel type, and operating conditions. Data on the potential health effects of DE inhalation are available mostly for what has been termed "traditional diesel exhaust," or TDE (2, 3, 14). TDE refers to the exhaust from diesel technology typical of the engines, fuel injection systems, fuels, and lubricants in use prior to 1988, when exhaust emissions from diesel were not regulated in the US.

Stimulated by progressively more stringent DE regulatory standards, major advances have occurred in diesel technology over the past two decades that have resulted in quantitative and qualitative changes in diesel-engine emissions, including dramatic reductions in emitted DEP, nitrogen oxides (NOx), and other components. Present-day engines emit "new technology diesel exhaust" (NTDE), where NTDE refers to the diesel exhaust from modern integrated systems (engines, fuel injection systems, ultra-low-sulfur fuels, lubricants, and exhaust after-treatment devices) manufactured and sold in the post-2006 era to meet the stringent US
Environmental Protection Agency (US EPA) emissions standards for heavy-duty highway diesel engines. Both DE from post-2006 diesel engines, as well as DE from engines retrofitted with a diesel particulate filter (DPF) and operated with ultra-low sulfur diesel fuel, are considered to be representative of NTDE, providing that they can achieve the 2007 US EPA emissions standard for DEP.

The US EPA-mandated changes in DEP and NO\textsubscript{x} emissions over time are illustrated in Figure 1. In addition to > 90% reductions in DEP mass, the chemical composition of the DEP and gases released from NTDE are qualitatively and quantitatively different from TDE (15, 16). With NTDE, emissions of DEP and NO\textsubscript{x} are over 99% lower than emissions from engines prior to regulations. Other aspects of NTDE are summarized elsewhere (15, 16). It is important to note that most of the available health-effects research does not provide accurate information for assessment of human health effects that might be associated with current and future exposures to NTDE.
Figure 1. Emissions reductions due to US EPA standards for DEP from heavy-duty diesel trucks (t) or urban buses (ub), relative to pre-1988 emissions. Panel A, DEP; Panel B, NO\textsubscript{x} (data from (1); figures adapted by permission from Informa Healthcare: Critical Reviews in Toxicology (2)).

Panel A

Panel B
The majority of our review thus relates to historical and present-day exposures to TDE, given that most of the available DE toxicological data are for studies of pre-2006 diesel engines lacking modern aftertreatment systems. TDE is composed of a gas-and-vapor phase, which is typically composed of about 98% carbon dioxide and water vapor, and also includes NOx, sulfur dioxide, carbon monoxide, and methane plus non-methane volatile organic carbon compounds. The particulate phase of TDE, i.e., DEP, from a traditional diesel engine running on high sulfur fuel typically represents less than 1% of the mass of total diesel exhaust emissions (17).

Exposures to TDE are generally described by giving only the DEP concentration, which may leave the erroneous impression that any observed health effects are due exclusively to the DEP alone. In fact, several studies have shown that the gaseous components of engine exhausts may be more important than the particulate components (18-20). In any case, DEP is often used as a surrogate measure for all the components in the DE mixture.

A major problem in estimating DEP exposure is that most areas where diesel engines are in use also include airborne particles from many other non-diesel sources, including carbon compounds from, e.g., tobacco smoke, gasoline engine exhaust, tire-wear dust, combustion of wood, paper and waste, meat-cooking fumes, hydrocarbon solvents, deposited and resuspended pollen, and asphalt dust. The exhausts of engines (both gasoline- and diesel-powered) share similar physical and chemical characteristics with each other and with airborne materials from many other combustion sources. There is no known marker for distinguishing DEP from other types of carbon-based dust. Thus, it has been difficult, if not impossible, to quantify the portion of an individual's total airborne PM exposure that derives from vehicle exhaust generally, and even more difficult to quantify the portion that is specifically related to DE. Indeed, DE and
gasoline engine exhaust exposures are generally present together and cannot be clearly separated (21).

US EPA has collected data on environmental concentrations of DEP derived from extrapolations of measured elemental carbon (EC) and other markers, and the agency has also generated estimates of DEP levels using a variety of source apportionment strategies (1, 22). The agency has estimated that U.S. long-term average urban and rural exposure concentrations from on-road vehicles and stationary sources range between approximately 0.2 and 3.0 µg/m³. Historically, DEP concentrations in occupational environments have been considerably higher than the ambient levels, as illustrated in Table 1.

Table 1. Approximate DEP concentrations in ambient and occupational environments where TDE is, or was, historically, present (9, 1, 23, 24).

<table>
<thead>
<tr>
<th>Exposure Environment</th>
<th>DEP (µg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term Average Ambient Levels</td>
<td>&lt; 0.2 – 3.0</td>
</tr>
<tr>
<td>Truckers</td>
<td>7 – 10</td>
</tr>
<tr>
<td>Railroad workers</td>
<td>40 – 70</td>
</tr>
<tr>
<td>US surface miners</td>
<td>90 – 100</td>
</tr>
<tr>
<td>US underground coal miners</td>
<td>500 – 700</td>
</tr>
<tr>
<td>US underground metal/mineral miners</td>
<td>800 – 1,000</td>
</tr>
</tbody>
</table>

For the general public, short-term DEP exposure levels can be considerably higher than long term average exposure levels, and in fact, many human controlled-exposure studies use
DEP exposure levels in the range of 100 to 300 µg/m³. The range of typical DEP concentrations for exposures to humans (both in the laboratory and ambient settings) is illustrated in Figure 2 (note logarithmic scale of DEP concentrations).

**Figure 2:** Examples of short-term and long-term DEP concentrations relevant to general public exposure (figure adapted by permission from Informa Healthcare: Critical Reviews in Toxicology (3)). The Fruin et al. (25) data (7 – 23 µg/m³) represent measurements made inside vehicles during highway driving. The McCreanor et al. (26) data (EC concentrations, 4 – 16 µg/m³) represent 2-hr averages during weekday periods along London's heavily trafficked Oxford Street. (Note logarithmic scale of DEP concentrations.)

### 2.0 Health Effects of Diesel-Engine Exhaust: Potential for Cancer Risk

In 1989, IARC (11) found *limited evidence* from studies of humans for carcinogenicity of DE (evidence primarily derived from studies of railroad workers). The IARC panel noted in the monograph that, in the epidemiologic studies they reviewed, there were no direct data for the workers' DE exposures; historical exposures were either assumed based on job title or were
estimated from more recent exposure assessments (11, p. 132). The IARC finding of *sufficient evidence* from animal studies for carcinogenicity of DE was based primarily on studies in which rats (but not mice or hamsters) developed tumors after lifetime inhalation of very high concentrations of DE (≥ 2,200 μg/m³ DEP). At the time of the 1989 IARC review, the Working Group was not able to consider the strong evidence that developed subsequently regarding the fact that the lung tumors in rats are due to a rat-specific lung-clearance-overload mechanism triggered by long-term elevated exposures to PM (2).

The US EPA reviewed the science regarding health risks to humans from DE in 2002, in their "Health Assessment Document for Diesel Exhaust." This document included an extensive analysis of "Carcinogenicity of Diesel Exhaust" (Chapter 7), with a primary focus on a possible link to lung cancer risk. The agency concluded that the weight of the evidence supports a "likely" role for DE in the risk of lung cancer, but assessment of the DE database led to the conclusion that neither the existing epidemiological studies nor the laboratory animal data were adequate to quantitatively predict potential human health effects from exposure to DE, or to link DE to increases in lung cancer (1, 2, 10). When evaluating the evidence for causal associations between DE exposure and cancers to sites other than the lungs, the US EPA concluded: "Quite a few studies have examined DE for other effects such as bladder cancer, leukemia, gastrointestinal cancers, prostate cancer, *etc*. The evidence for these effects is inadequate" (1, pp. 7-80).

The Institute of Medicine of the National Academies also reviewed the science regarding health risks to humans from fuels and fuel combustion products (7). Although the IOM did not
draw a conclusion specific to DE, it did offer the conclusion that: "there is sufficient evidence of an association between exposure to combustion products and lung cancer."

Of the occupations listed in Table 1, truckers and railroad workers have been the focus of most epidemiology studies regarding DE-exposed populations. A significant problem with the data on truckers is that increases in lung cancer were seen in this occupation prior to dieselization (See Table 9 in (2)). As discussed subsequently, studies of underground miners have generally been negative for lung cancer risk. Consequently, U.S. railroad workers have formed the basis of most studies regarding DE-exposure and its possible relationship to lung cancer risk. These include case-control and retrospective cohort mortality studies reported by Garshick and collaborators (27-30) and a reanalysis of the epidemiologic data by Crump (31). The railroad epidemiologic studies were based on computerized work records maintained since 1959 by the U.S. Railroad Retirement Board (RRB). By 1959, the transition from coal-powered to diesel-powered locomotives was 95% complete in the U.S. However, one of the issues in the railroad worker data is that the lung cancer mortality results do not show a clear trend with increasing DEP concentration when the cohort is examined by job category. This is summarized in Table 2, and additional details are available in Hesterberg et al. (2).
Table 2: Railroad-worker personal exposures, by job category, to estimated DEP \(^a\) \((n = \text{number of measurements})\) versus lung cancer mortality relative risk \((n = \text{number of lung cancer deaths})\), based on U.S. lung cancer mortality rates \((31, 32)\).

<table>
<thead>
<tr>
<th>Job Category</th>
<th>Exposure</th>
<th>Lung cancer mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Estimated DEP (^a) (\mu g/m^3), geometric mean</td>
</tr>
<tr>
<td><strong>Unexposed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clerks</td>
<td>59</td>
<td>17</td>
</tr>
<tr>
<td>Signal men</td>
<td>13</td>
<td>49</td>
</tr>
<tr>
<td><strong>Exposed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engineers/Firemen</td>
<td>128</td>
<td>39 – 73</td>
</tr>
<tr>
<td>Conductors/Brakemen</td>
<td>158</td>
<td>52 – 92</td>
</tr>
<tr>
<td>Hostlers</td>
<td>8</td>
<td>191</td>
</tr>
<tr>
<td>Shop Workers</td>
<td>174</td>
<td>114 – 134</td>
</tr>
</tbody>
</table>

\(^a\) For each air sample, estimated fraction of cigarette smoke was subtracted from total respirable particulate matter; adjusted data are presumed to reflect the concentration of airborne DEP \((32)\).

\(^b\) Clerks and signal men combined.

\(^c\) Conductors/brakemen and hostlers combined.

In the late 1990s, the Health Effects Institute (HEI) convened a "Diesel Epidemiology Expert Panel" \((33)\) to review the railroad worker data and the various analyses of these data. The Panel was assisted by leading investigators who had conducted epidemiologic research on DE, and by expert analysts who had conducted critical reviews of that research. The Panel noted that lung cancer mortality for train workers/riders (engineers, conductors) was higher than for clerks/signalmen (unexposed), with shop workers (highest DEP exposure) exhibiting intermediate risks (See Table 2, above). However, the Panel's analyses also demonstrated that within each category of worker, the risk of lung cancer decreased with increasing duration of employment, and, further, that the decrease was statistically significant for clerks/signalmen and train workers. HEI's Diesel Epidemiology Expert Panel concluded that the data: "are not
consistent with a steadily increasing association between cumulative diesel exposure and lung cancer risk." The Panel noted that the negative exposure-response relationship could be due to a number of possible reasons, including unmeasured confounding variables and/or "other sources of pollution" that could have been responsible for the observed effect. The HEI Panel also found that the conclusions initially reached by Garshick and co-workers (i.e., a positive exposure-response relationship) resulted entirely from baseline differences in risk between the two job categories (train workers versus clerks/signalmen). The Panel concluded: "These patterns are not consistent with a monotonically increasing association between cumulative exposure to diesel exhaust and lung cancer risk. If risk increased consistently with increasing exposure, a positive trend with duration of employment would be expected for the exposed groups (including train workers), even if exposure magnitudes were incorrect." That is, the weight of evidence suggested that, in the railroad-worker studies, any occupational increase in lung cancer risk among train workers was not due to DE exposures.

Laden et al. (34) published a report that used historical data on dieselization of individual railroads and emission factors for locomotives in each railroad for an innovative exposure intensity characterization. These investigators calculated annual railroad-specific probabilities of worker exposure to DE, going back to 1945. As expected, RRs for lung cancer remained elevated, but there was no evidence of an exposure-response relationship using these improved measures of exposure intensity. Another innovative approach to RR-worker data is a case-control analysis of lung-cancer mortality (1981-1982) by Lee et al. (35), using a new type of Kaplan-Meier survival plot in which the time scale represents a measure of disease progression rather than mortality over calendar time. The authors reported that workers who had been
engineers or brakemen had a better initial health status, but a steeper rate of health decline than workers who had never been engineers or brakemen. This steeper rate of decline was attributed by the authors to DE exposure, however, no quantitative measures of DE exposure were used other than the "job categories" from the original study (28), which were shown to be problematic in the HEI (33) analysis.

In a coal-dust analysis relevant to DEP, IARC reviewed studies of coal miners and determined that evidence was inadequate to show any lung cancer risk from coal dust exposure; IARC classified coal dust as Group 3, i.e., "cannot be classified as to its carcinogenicity to humans" (36). This conclusion is important with regard to DEP for two reasons: (1) The studies in the IARC review included coal miner cohorts of workers who historically inhaled some of the highest occupational concentrations of total airborne particulates among all working environments, and this potential "lung overlead" did not lead to lung cancer in humans as it does in rats. (2) Hundreds of underground coal mines, particularly outside the US, had used diesel equipment for many decades, exposing those coal miners to considerable DEP as well as to coal dust. As illustrated in Table 1, and stated in US EPA's HAD for diesel exhaust, "The highest occupational exposures to DEP are for workers in coal and non-coal mines using diesel-powered equipment" (1, pp. 2-107). Yet, as IARC found, lung cancer did not appear to be elevated.

Studies of underground miners have been recently reviewed by Hesterberg and colleagues (2), and the results indicate that between the high levels of DEP exposure and the adequacy of latency periods, lung cancer risk, if any, should have been readily identified. That is, the underground mining studies are potentially much more powerful than studies of other
occupational groups. However, while not definitive, the underground miner studies provide no convincing evidence of a risk of lung cancer associated with DEP, and are generally consistent with no increased risk. Thus, even with DEP exposures that were an order of magnitude higher than those in either the trucking or railroad industries studies, the mining studies are, on balance, consistent with no detectable increase in risk.

For more than 10 years now, a long-standing collaboration between the National Institute of Occupational Safety and Health (NIOSH) and the National Cancer Institute (NCI) has been evaluating mortality from lung cancer and other diseases among U.S. nonmetal miners in relation to quantitatively measured exposure to DE, using a cohort and nested case control study (37). NIOSH researchers (38, 39) just recently reported findings characterizing current and past levels of surrogates of diesel exhaust, deriving historical estimates of exposure to respirable elemental carbon (REC). These exposures are being used to investigate potential exposure-response relationships with lung cancer, but as of this writing (late 2010), no epidemiologic results have been published by NIOSH or NCI.

Laboratory studies of exposure to TDE, both *in vivo* and *in vitro*, have limited relevance in assessing the carcinogenic potential of TDE in humans. Laboratory rats, when exposed for a lifetime to high levels of TDE (> 2,200 μg/m³), develop excess lung tumors; however such lung tumor increases are consistently observed in rats exposed to overload levels of other types of fine particles (TiO₂, talc, and carbon black). Other species (mice and hamsters) exposed at high DEP levels do not show an excess of lung tumors, nor do rats exposed at lower DEP levels. In rats, prolonged and elevated exposures to a variety of different particulates result in lung overload,
lung inflammation, lung epithelial cell proliferation, and eventually lung tumors. This mechanism is not DEP-specific and did not occur in the rats at DEP exposure concentrations below 600 μg/m³ (40), a concentration level that is 10-fold greater than TDE DEP levels to which railroad and trucking industry workers might have been exposed, and 100-fold above ambient concentrations. Thus, the tumorigenic effect of high levels of TDE in rats is considered to be a non-specific particle effect that results from a unique rat-lung overload mechanism (14, 41, 42). In fact, if any occupation might have resulted in "lung overload" in humans, underground coal miners might be the most likely in this regard. As described earlier, IARC did not find evidence of lung cancer risk among coal-miner populations. Thus, the rat "lung overload" phenomenon should not be considered relevant to humans exposed either to DEP in occupational environments or to even lower ambient levels.

A final aspect of the laboratory-animal studies relates to the potential mutagenicity of DEP organic extracts, and reviews of these data are available elsewhere (2, 8, 43, 44). Although the organic compounds extracted from DEP using strong solvents like dichloromethane are capable of damaging DNA in vitro, such damage has not been established to occur under the conditions of the DEP inhalation bioassay. Moreover, DEP organic compounds are poorly bioavailable (12), and the fact that lung tumors can be induced in rats exposed by lifetime inhalation of non-DEP particles with virtually no adsorbed organics (e.g., carbon black, titanium dioxide) supports the conclusion that PM overload per se, and not the organics bound to DEP, are responsible for lung tumors in rats exposed to overloading levels of DEP.
Conclusions on Potential Cancer Risks of Inhaled DE

A number of careful analyses of the DE epidemiologic data have concluded that existing epidemiological studies are unable to quantitatively link DE exposure levels to increases in lung cancer (2, 6, 33, 45-48). Several factors that support this conclusion are: (1) the epidemiologic database lacks consistency, with some studies showing a weak association between presumed DE exposure and lung cancer and other studies showing no association; (2) there is an overall lack of dose-response among DE-exposed populations, with truckers at low exposures showing greater risks than underground miners at high DE exposures (49); (3) some studies show negative exposure-response relationships, and for those with weak positive associations with DE exposure, the result may be attributable to residual confounding (particularly by smoking); (4) many of the DE epidemiologic studies suffer from inadequate latency periods; (5) given the negative mutagenicity data on DEP per se, and a negative animal database (aside from lung-overloaded rats), biological plausibility remains an issue; and (6) the epidemiological studies lack adequate DEP exposure information, without which scientists cannot establish a quantitative estimate of human cancer risk.

3.0 Health Effects of Diesel-Engine Exhaust: Potential for Non-Cancer Health Effects

Laboratory-Animal Data

Numerous non-cancer health endpoints for DE have been investigated in animal studies. Compared with observational and experimental human studies, laboratory animal studies offer several unique advantages, including the study of both healthy and diseased animals (e.g.,
spontaneously hypertensive rats, atherosclerotic mice, lung-compromised mice) over a range of well-defined exposure levels and controlled study conditions. Importantly, laboratory studies allow for the assessment of serious health impacts, including longer-duration, rare, and permanent health effects as well as those that require invasive measurement methods (e.g., multi-organ pathology), not possible in human studies. Through the use of elevated exposure levels, large numbers of study animals, sensitive animal species, and animal models of disease, laboratory animal studies can be designed to increase the likelihood of detecting adverse effects from DE inhalation. In fact, sometimes specific genetic strains are used to discover possible effects of DE on, say, resistance to infection (50) or endothelial function (51). However, extrapolation of conclusions derived from animal species to humans can be highly uncertain given that laboratory experiments are typically conducted at extremely high exposure levels, experimental routes of administration may differ from physiological, real-life exposures (e.g., intratracheal instillation is not representative of real-life inhalation exposures), and there may be large interspecies differences in sensitivity and mode of action.

The 2002 US EPA "Health Assessment Document for Diesel Engine Exhaust" (Diesel HAD) examined evidence regarding potential non-cancer health effects of both long-term and short-term exposure to DE and DEP. In terms of elevated, short-term exposures, the agency stated that DE at sufficient levels can cause transient irritation, inflammatory symptoms, and may exacerbate asthma symptoms. For long-term exposures, US EPA identified a DEP reference concentration (RfC), representing a lifetime exposure concentration not expected to cause adverse non-cancer health effects, of 5 μg/m³ (1). This RfC was based on exposure-response data for inflammatory and histopathological changes in lungs of rats exposed to high
levels of DE over extended periods of time. However, as noted earlier, as cleaner NTDE engines replace a substantial number of existing, traditional diesel engines, the applicability of the US EPA RfC analysis will need to be reevaluated.

With regard to non-cancer effects of DEP exposure, Hesterberg et al. (3) provide a critical evaluation of over 100 published articles on experimental research. It is important to note that even recent studies continue to use DE emissions from a wide variety of old, traditional diesel engines and fuels, and hence the results likely have limited relevance to the modern fleet of vehicles emitting new technology diesel exhaust (NTDE). For example, out of all of the DE studies, very few have used DE from an engine running on diesel fuel meeting the 2007 US EPA standard, and most other studies have used engines or fuels no longer commercially available. Also, animal studies were often carried out at elevated TDE concentrations, sometimes reaching DEP levels as high as 20,000 to 100,000 μg/m³.

As reviewed by Hesterberg et al. (3), several large-scale experiments with animal species inhaling DEP have been completed since the US EPA Diesel HAD (1). These data show that highly elevated DEP doses (DEP concentration x exposure duration) can lead to chronic lung inflammation, but that at smaller doses, still much higher than typical ambient levels of DEP, little has been reported in the way of adverse or irreversible effects. In fact, one group of investigators suggested a no-observable-adverse-effect level (NOAEL) in the range between 200 to 1,000 μg/m³ DEP (52), and other investigators point out that rat exposure levels turn out to be roughly equal to human exposure concentrations (HEC) (within a factor of 2) by taking into account dose per lung (alveolar) surface area, relative lung deposition, and relative lung
clearance (53). Thus, more recent data, subsequent to US EPA setting the RfC at 5 μg/m³, suggest that the 5 μg/m³ is quite conservative and health protective.

The McDonald et al. (17) laboratory animal study provides some of the few available experimental data for a diesel exhaust mixture representative of NTDE. This study investigated the relative toxicity of acute inhalation exposures (6 hrs per day over 7 days) for a baseline uncontrolled, TDE emissions case (approximately 200 μg/m³ DEP) versus an emissions-reduction, NTDE case (low sulfur fuel, catalyzed ceramic trap, near background levels for all emissions but NOₓ) on a suite of sensitive measures of acute lung toxicity in mice, including lung inflammation, RSV resistance, and oxidative stress. For the baseline TDE case, McDonald et al. (17) observed statistically significant DE-induced effects for each class of responses, while these effects were either nearly or completely eliminated for the NTDE case.

With its well-characterized exposure atmosphere and its assessment of a suite of sensitive measures of acute lung toxicity, the McDonald et al. (17) study provides some of the strongest experimental evidence of the reduced toxicity of NTDE. However, as a single study that addressed only acute lung toxicity in a single animal species for a single engine and aftertreatment configuration, there is clearly a need for more comprehensive toxicological investigations of NTDE to confirm these findings of reduced NTDE toxicity for a broader range of aftertreatment configurations and operating conditions and for other classes of health endpoints (e.g., cardiovascular effects, allergic effects). On this note, it is expected that the comprehensive health effects components of the ongoing Advanced Collaborative Emissions Study (ACES) will provide a wealth of information for assessing the carcinogenicity, subchronic,
and chronic non-cancer toxicity of NTDE. A core component of ACES is a chronic rat bioassay where rats are exposed via inhalation for 24 or 30 months, with interim sacrifices at 1, 3, 12, and 24 months, to three dilutions of whole emissions from a 2007-compliant diesel engine with advanced emission control technologies (and clean air controls). Health endpoints of interest include not only carcinogenicity but also pulmonary function, pulmonary inflammation, oxidative damage, lung cell proliferation, histopathological changes, and hematological effects.

*Exposure of Human Volunteers to DE*

Controlled-exposure studies with human volunteers have several major advantages, including the direct study of human subjects; the use of well-defined, continuously measured exposure concentrations and durations; the absence of confounding exposures to other chemicals; and the use of sophisticated research equipment to simultaneously and precisely measure a suite of physiological endpoints potentially relevant to health, including both subtle biological responses and small functional decrements. Although exposures via nasal masks or mouthpieces have been used in some studies, large state-of-the-art inhalation chambers (*e.g.*, 25 to 100 m$^3$ whole-body chambers with control of humidity, temperature, ventilation, *etc.*.) are currently used, wherein small numbers of volunteers receive whole-body exposures to one or more pollutants while either exercising or at rest. Some controlled human exposure studies have been performed using adults representative of potentially susceptible subpopulations, including those with asthma, chronic obstructive pulmonary disease, and cardiovascular disease.
Studies on human volunteers also have limitations. Namely, (1) small groups of healthy volunteers may not be representative of larger populations; (2) investigation of only acute effects from short-duration exposures may not extrapolate to long-term exposures; and (3) use of exposure protocols and pollutant concentrations expected to elicit only transient, clinically mild health responses may not extrapolate to higher-level, worker exposures (54, 55). In addition, there is generally no control of the recent exposure history of volunteers, which can be an important modifier of study findings. To increase the likelihood of detecting effects among small groups of healthy subjects, human exposure studies often use relatively high DE concentrations, compared to actual real-world exposure levels, yet, of course, below those that are suspected to increase the risk of long-term or irreversible health effects. Because of these limitations, human controlled exposure studies may be underpowered to detect some health responses that are of health significance.

Because of the mild nature of the responses measured (often with subjects reporting no perceptible symptoms), the question arises as to whether the response is an "adverse health effect." Although physiologic impairment or clinically significant decrements may reach adverse levels, the American Thoracic Society (56) has pointed out that not all "effects" measured in clinical-exposure studies reflect "adverse health" outcomes warranting regulatory, preventive measures. Specifically, ATS (56) concluded that "not all changes in biomarkers related to air pollution should be considered as indicative of an injury that represents an adverse effect." Similarly, ATS (56) concluded that "a small, transient loss of lung function, by itself, should not automatically be designated as adverse." ATS (56) further distinguished adverse
symptoms as those that are associated with "a diminished quality of life or with a change in clinical status."

Since the preparation of the Diesel HAD, a number of new human controlled exposure studies have been published, with several of these studies following up on previous studies of lung and systemic inflammatory responses, and other recent studies addressing cardiovascular health responses including cardiac, vasomotor, and fibrinolytic function (3). Studies of human volunteers have used elevated concentrations of DE (about 100 to 300 μg/m³), markedly above what would be experienced in typical non-occupational environments. An illustration of the recent cardiovascular-related results that have been found in humans is given in Figure 3, where the vertical axis gives the concentration of DEP, and the horizontal scale lists the type of studies that have been done. Cardiovascular health responses have been a focus of a number of recent DE human clinical studies, and as shown in Figure 3, these studies have generally reported mixed findings with respect to various subclinical measures of acute cardiovascular health responses. However, none of these studies have examined NTDE, the low emission technology that is required for all diesel on-road vehicles starting in 2007. In fact, preliminary data are available from two studies (57, 58) showing a reversal of effects on vasomotor function and thrombus formation with addition of a commercially available retrofit particle trap to the test heavy-duty diesel engine.
**Figure 3:** Graphical summary of key findings from recent controlled human exposure studies of the cardiovascular-related health effects of inhaled DE (both particles and gases), with Panel A addressing systemic inflammatory and fibrinolytic/thrombogenic responses and Panel B showing effects on subclinical markers of cardiovascular disease (e.g., heart rate, blood pressure, heart rate variability, etc.), effects on vascular function, and changes in indicators of endothelial dysfunction. Green circles with – signs represent negative (null) findings, red squares with + signs represent positive findings, and yellow triangles represent equivocal findings.

**Panel A**
Panel B

Overall, given the relatively small number of statistically significant DE-induced changes observed in these recent human-exposure studies for some test parameters among a larger number of non-significant findings, as well as some conflicting findings among studies (Figure 3), it remains difficult to interpret the broader health implications of what currently remains a disparate body of findings from studies of small numbers of subjects and different study designs and measurements. Although animal studies have provided some findings suggestive of
potential mechanisms (e.g., the Wong et al. (59) findings suggesting a neurokininergic mechanism for DE-induced lung inflammation), our mechanistic understanding remains limited. This is particularly true for low-level ambient DE concentrations, given that most animal studies have been conducted at concentrations vastly higher than those used in controlled human exposure studies. Broader interpretation of these human clinical data is limited by the fact that all but a few of the available studies were conducted in the same exposure laboratory for freshly-generated emissions from the same engine type under the same operating conditions (idling Volvo TD45, model year 1991, 4.5-liter, 4 cylinder, 680 rpm, diesel engine). This places limits on generalizing the study findings to emissions from other engines and operating conditions, and to environmentally-aged diesel emissions. As summarized in the 2002 Diesel HAD (1) and Ris (60), it is well-established that DE emissions vary both in chemical composition and particle size distributions for different types and ages of diesel engines, and within engine types, depending on operating conditions (fuel composition, load characteristics, lube oil composition, emission control devices), with atmospheric aging also serving to modify DE properties.

The question regarding potential health effects of "aged" DE emissions might be best addressed by studies such as that of McCreanor et al. (26), where both health effects and PM levels were assessed for 2-hour weekday exposure periods between November 2003 and March 2005 along London's heavily-trafficked Oxford Street (Figure 2, EC concentrations, 4 – 16 μg/m³, median PM$_{2.5}$ concentration 28 μg/m³). The Oxford Street location was chosen to represent a busy city street impacted by diesel-vehicle emissions, because only diesel-powered buses and taxicabs are permitted on this heavily-trafficked street, while Hyde Park (EC concentrations, 0.4 – 7 μg/m³, median PM$_{2.5}$ concentration, 12 μg/m³) is a traffic-free area of
London. Ultrafine-particle concentrations, in terms of 1,000's of particles per cm$^3$, were 64 on Oxford Street and 18 in Hyde Park.

The McCreanor et al. (26) study included 60 participants with mild to moderate asthma, and each participant walked for 2 hours along a heavily trafficked street (Oxford Street) and, on a separate occasion, through a nearby, traffic-free park (Hyde Park). The investigators observed small, statistically significant, "asymptomatic but consistent" reductions in FEV$_1$ (up to 6.1%) and FVC (up to 5.4%) for the Oxford Street exposures compared to those for the Hyde Park exposures. These mild, reversible lung function changes for the Oxford Street exposures coincided with statistically significant increases in sputum myeloperoxidase levels, a biomarker of neutrophilic inflammation, and airway acidification. However, between the two sites, there were no statistically significant changes in FEF$_{25-75\%}$, mean FE$_{NO}$, sputum neutrophil counts, interleukin-8, sputum eosinophil counts, or eosinophil cationic protein levels. Using pollutant-specific, exposure-response analyses, the study investigators linked the observed health responses with the significantly greater air pollutant exposure concentrations measured during the Oxford Street exposure period (e.g., 5.7-fold higher EC concentrations and 3.5-fold greater ultrafine particle count concentrations), noting that, in two-pollutant models, the effects of ultrafine particles and elemental carbon retained statistical significance and were most consistent. Although the study authors suggest elevated exposures to DE on Oxford Street may underlie their study findings, they do acknowledge the possibility that other unmeasured pollutants associated with roadside diesel-traffic exposure, namely road dust and engine or tire debris, may instead be the causal agents. Likewise, noise, temperature (2°C hotter on Oxford Street), and other stresses may have also played a role in the measured differences.
Although not specific to DE, a number of observational epidemiology studies have linked cardiovascular and respiratory health effects to traffic-related air pollution (74-76). The HEI Panel on the Health Effects of Traffic-Related Air Pollution (76) conducted one of the more comprehensive reviews of the health effects literature related to traffic-related air pollution, concluding that "the evidence is sufficient to support a causal relationship between exposure to traffic-related air pollution and exacerbation of asthma." In addition, the HEI panel concluded that there was "suggestive evidence of a causal relationship" with onset of childhood asthma, nonasthma respiratory symptoms, impaired lung function, total and cardiovascular mortality, and cardiovascular morbidity. Importantly, studies of traffic-related air pollution have generally relied upon surrogate measures of exposure to traffic-related pollution, including both traffic-pollutant surrogates (e.g., CO, NO₂, EC) and proximity-based surrogates (e.g., distance of residence to nearest road, and traffic density). As a result, the role of DE and DEP in these associations cannot be clearly assessed due to the non-specificity of the pollutant surrogates, and due to many potential confounding factors associated with surrogates based on proximity, such as gasoline-engine exhaust, noise, fugitive road dust, socioeconomic status, and so forth (77). Furthermore, as acknowledged in HEI (76), the available epidemiology studies are primarily for past time periods and older vehicles, meaning that they are of uncertain, and possibly little, relevance to today's modern engine technologies and fuels.

*Conclusions on Potential Non-Cancer Health Effects of Inhaled DE*
Human controlled-exposure studies provide evidence suggestive of lung inflammatory effects and thrombogenic and ischemic effects of inhaled DE, albeit for older model diesel engines and concentrations that are much higher (~300 μg/m$^3$) than typical ambient or even occupational levels. Recent animal studies provide insights on the potential mechanisms underlying observed respiratory and cardiovascular health responses, but because of unrealistically high DE concentrations, it is unclear whether the mechanisms elucidated in these studies are relevant at lower DE exposure levels. A mechanism of action that allows reliable prediction of adverse health impacts at DE exposure levels typical of the present-day ambient and occupational environment has not emerged. Because of changing diesel-engine technology, inhalation studies using realistic environmental and occupational exposures of new technology diesel exhaust (NTDE) are of critical importance.

4.0 Conclusions

Much remains to be understood in terms of the constituents of TDE and NTDE that have the greatest potential for eliciting health effects at adequately high exposure concentrations and durations. There are important differences in mass emission rates, particle size distributions, elemental carbon vs. organic carbon content, ratios of 3- and 4-ring polycyclic aromatic hydrocarbons (PAHs), and other constituent concentrations, not only between TDE and NTDE, but also among various types of engines within the two classes (15, 16). In fact, it cannot be clearly stated whether DE is of greater or lesser toxicity than other combustion sources such as gasoline-engine exhaust, wood smoke, fossil-fuel power plants, compressed-natural-gas engines, and other combustion sources. These issues are crucial both for DEP and ambient PM, where there is disagreement between observational epidemiology and toxicology as to the sufficient
dose and toxic role of chemical constituents and physical characteristics causing biologic activity.

Although DE-exposed worker populations have been studied a great deal, both inadequate estimates of DE exposure as well as poorly-understood exposure-response relationships have prevented development of quantitative estimates of lung-cancer risk. In fact, there does not appear to be an exposure-response relationship over different occupations that have considerably different exposure potential for DE. A high priority is the reporting of results from the longstanding NIOSH/NCI studies of US underground miners with historically elevated DE exposures. These results need to be completed, analyzed, and reported in detail in the open literature.

Emissions of DEP nanoparticles (i.e., with diameters < 100 nm) have received increased attention in recent times, due to reports that nanoparticle emissions could be increased in NTDE, as well as due to the intense interest in the toxicity of engineered nanoparticles. Nano-sized particles, including individual nuclei mode particles and carbonaceous agglomerates, are well known to dominate particle number concentrations of DEP (78). Hesterberg et al. (5) examined findings from the DE human clinical studies to identify insights relevant to potential NP human health hazards, concluding that the available DE human clinical data do not give evidence of a unique toxicity for NPs (at least in the DEP context) as compared to other small particles. Given that particle number and surface area concentrations are recognized as more relevant exposure metrics for nanoparticle health effects than particle mass concentration, there is a real need for DE health effect studies to quantify these exposure metrics (in addition to mass concentrations).
Recent studies of DE exposures to human volunteers have provided useful human evidence of the potential respiratory and cardiovascular health hazards of short-term exposures to elevated DE exposures, but there is a need to better understand the human clinical significance of these findings and their relevance to lower, ambient levels of DE and a wider range of engine types, operating conditions, and population subgroups. In addition, there is a need for more comprehensive toxicological investigations of NTDE to confirm the toxicological differences between NTDE and TDE indicated by the distinctions in their chemical and physical characterization properties. Furthermore, with recent research indicating that other combustion emissions (e.g., gasoline engine emissions) and ambient particle types can elicit similar biological responses as DE, further study is also needed to understand how DE health risks compare to those from other common air contaminants. With further study and improvements in our understanding of not only DE hazard assessment, but also dose-response for realistic inhalation exposures, it can be hoped that newly-collected data will stimulate continuing progress toward a cleaner-burning, increasingly safer diesel engine technology.
References:


16. Hesterberg TW, Long CM, Sax SN, et al. Particulate matter in new technology diesel exhaust (NTDE) is quantitatively and qualitatively very different from that found in traditional diesel exhaust (TDE). 2011; *Submitted to the Journal of the Air & Waste Management Association*.


