Special Report 16 November 2007

Mobile-Source Air Toxics: A Critical Review of the Literature on Exposure and Health Effects

Summary of Studies of Diesel Exhaust

For further information please contact the Health Effects Institute:
+1-617-886-9330
pubs@healtheffects.org
www.healtheffects.org

Summary of Studies of Diesel Exhaust

INTRODUCTION

Because numerous comprehensive reviews of the health effects of exposure to diesel exhaust (DE) have been published, the panel elected not to review the literature on diesel particulate matter (DPM) or diesel organic gases again in this special report. Instead, the following expanded summary of DE research has been provided.

DE is a complex mixture consisting of particulate-phase particulate matter (PM) and thousands of gaseous organic and inorganic components. DPM and diesel organic gases are designated as MSATs by the EPA. DPM is an agglomeration of nonvolatile elemental carbon particles with adsorbed semivolatile polycyclic organic matter (POM), metals, and sulfate ions. The size distribution of DPM is generally bimodal, with an accumulation mode, which accounts for most of the PM mass, and a nuclei mode, which accounts for most of the PM number (EPA 2002a; Kittelson et al. 2002; Sakurai et al. 2003). The PM in accumulation mode consists largely of soot (solid carbonaceous material and ash) and adsorbed organic and sulfur compounds and ranges from 30 to 1000 nm. PM in the nuclei mode consists of particles composed largely of the volatile organic and sulfur compounds and small amounts of solid, metallic compounds that are generally smaller than 30 nm (EPA 2002a; Kittelson et al. 2002). However, the boundary between the two modes can vary (Kittelson et al. 2002; Sakurai et al. 2003).

Several other MSATs are found in DE, including acetaldehyde, acrolein, benzene, formaldehyde, POM, and chromium compounds (EPA 2002a). DE contributes to ambient concentrations of PM, nitrogen oxides (NO_x, a component of smog), and ozone (also a component of smog—formed as a result of atmospheric reactions of semivolatile and volatile organic hydrocarbons and NO_x). Because other combustion products (such as smoke from burning wood and coal and industrial processes) also contribute to the concentrations of these pollutants to varying degrees in various places, it is difficult to assess exposure to DE in the general population. Allowable ambient concentrations of PM, nitrogen dioxide (NO_2), which is the most abundant species of NO_x, and ozone are set by U.S. National Ambient Air Quality Standards (U.S. Congress, House of Representatives 1977).

As part of an effort to reduce ambient PM and NO_x, the emission rates of these pollutants from diesel engines, as well as the emission rates of hydrocarbons and carbon monoxide, have been regulated since 1977. Regulatory efforts have historically been aimed at the heavy-duty diesel engines used in on-road trucks and buses. Beginning in 1996, efforts were broadened to include non-road equipment (EPA 1998b; EPA 2004b) and, beginning in 2000, railroad locomotives (EPA 1998c). In addition, effective as of 2004, PM and NO_x standards required both light-duty vehicles and diesel-fueled vehicles to meet the same emission standards as gasoline-fueled vehicles (EPA 2000a). Substantial additional reductions in PM and NO_x emissions are mandated for heavy-duty 2007-to-2010 on-road vehicles (EPA 2001a). These reductions require more advanced emission controls, such as catalyzed PM filters, exhaust-gas recirculation, and NO_x adsorbers, or selective catalytic reduction. Both the size and composition of the PM emitted by 2007-compliant engines are expected to be substantially different from those of earlier engines. The accumulation mode (particle mass) would be very low. Engine exhaust would consist largely of volatile or semivolatile nuclei-mode particles (Vaaraslahti et al. 2004). Certain particle filters might increase the NO_2:NO ratio in exhaust (Carslaw and Beevers 2004; Kittelson et al. 2006). PM emissions from 2010-compliant engines will be similar to those from 2007-compliant engines, but NO_x emissions will be substantially lower. However, the advanced NO_x-control technologies that will be used (such as the NO_x adsorbers and selective catalytic reduction) might generate new, potentially toxic chemical species.

The studies summarized here pertain mainly to the health effects of exposure to emissions from older or recent diesel engines. Although the expectation is that new diesel engines will contribute much less pollution to ambient air, it is still important to evaluate the exhaust from these engines, in particular to ensure that possible new emission species will not cause new adverse effects on human health.

A glossary of terms appears on page 17; a list of abbreviations and other terms appears at the end of this report.

KEY LITERATURE REVIEWS

This expanded summary of the literature on exposure and health effects of DE is based on a review of original studies as well as reviews of the literature conducted by HEI (HEI Diesel Working Group 1995, HEI Diesel Epidemiology Expert Panel 1999), the California EPA (1998), and the EPA (2002a).

EXPOSURE SUMMARY

Because of the prevalence of diesel-fueled engines, DE and its by-products are present in most ambient environments. Urban and industrial areas—especially near roadways, truck and bus depots, and construction sites—typically have higher concentrations of DE pollutants (California EPA 2003). Current estimated ambient concentrations of DPM range from 3 to 10 $\mu g/m^3$; typical occupational concentrations range from 10 to 100 $\mu g/m^3$; the highest concentrations range from 100 to 1000 $\mu g/m^3$ and are found in poorly ventilated underground mines (EPA 2002a). Over the years, improvements in diesel fuel, such as decreases in sulfur content, and in diesel engines have reduced emissions of DE and many of its components (Gertler et al. 2002). Thanks to new regulations, this trend is expected to continue. Diesel engines designed to meet the 2007 emission standards will emit only very small quantities of PM and elemental carbon. Over a period of many years, these engines will gradually replace older engines. Retrofitting of the oldest diesel vehicles with PM filters will also contribute to emission reduction (LeTavec et al. 2002; Lanni 2003).

HEALTH EFFECTS AND REGULATORY SUMMARY

GENOTOXICITY

Since the late 1970s, extensive testing of the genotoxicity of DPM (and fractions of DPM) has been conducted using both in vitro assays (of bacterial and mammalian cells) and in vivo assays. The premise for conducting these studies was that the carcinogenic responses seen in earlier animal studies might have been caused by various organic compounds adsorbed to the particles (particularly polycyclic aromatic hydrocarbons [PAHs] and their nitrogen derivatives), many of which are mutagenic in in vitro assays (EPA 2002a). Most of the in vitro tests involved resuspended DPM or DPM extracts rather than whole DE.

The in vitro and in vivo assays used to evaluate the genotoxicity of DPM and particle extracts have been reviewed by Shirnamé-Moré (1995), the EPA (2002a), and the California EPA (1998). They found evidence of the following:

- Mutagenicity in bacterial assays (*Salmonella typhimurium*) and in mammalian cell cultures in the presence or absence of exogenous metabolic activation.
- Chromosomal damage in mammalian cell culture assays, measured as sister-chromatid exchanges (exchange of DNA between two chromatids of a chromosome), chromatid gaps and breaks, and formation of micronuclei (acentric chromosome fragments found in the cytoplasm). These changes have also been detected in bone-marrow cells of mice exposed to whole DE.
- Unscheduled DNA synthesis (a measure of the rate of DNA repair) measured either in cultured cells or in lung tissue of rodents exposed to whole DE.
- Small increases in DNA adducts in the lung tissue of rodents exposed to whole DE in some of the earlier diesel-inhalation studies but not in subsequent ones. Increases in DNA adducts were observed in workers exposed to DE (Hou et al. 1995; Hemminki et al. 1994). However, HEI's Diesel Working Group concluded that the adducts were not specific for diesel exposures (HEI 1995)

Regulatory Summary

The EPA (2002a) has reviewed the existing genotoxicity evidence in its *Health Assessment Document for Diesel Engine Exhaust* and concluded that "studies with *Salmonella* have unequivocally demonstrated mutagenic activity in both particulate and gaseous fractions of DE" and that "the induction of gene mutations and structural chromosomal aberrations have been reported in several mammalian cell lines after exposure to extracts of DPM." The EPA recognized, however, that "no single assay should be expected to either qualitatively or quantitatively predict rodent carcinogenicity."

The California EPA *Health Risk Assessment for Diesel Exhaust* (1998) also concluded that "diesel exhaust particles or their extracts are mutagenic in bacteria and several mammalian cell systems" and "induce chromosomal aberrations, aneuploidy and sister-chromatid exchange in rodent and human cells in culture."

These assays measure processes that might be relevant to some aspects of carcinogenesis, and agencies that assess risk have generally viewed them as useful for hazard identification.

CARCINOGENICITY

Animal Studies

The potential carcinogenic effects of DE from pre-1990 engines have been extensively investigated in long-term bioassays in rodents. The rat studies were conducted principally using exhaust from light-duty engines and showed an increase in lung tumors only at high exposure concentrations (several mg/m^3 DPM) (Heinrich et al. 1986, 1995; Ishinishi et al. 1986; Iwai et al. 1986, 1997; Mauderly et al. 1987, 1994; Brightwell et al. 1989). Only the rat study by Ishinishi and colleagues (1986) used exhaust from a heavy-duty engine (as well as a light-duty engine). All these engines had much higher emissions of DPM and gases than current and future diesel engines.

Two of the rat studies compared the carcinogenicity of whole DE with that of carbon-black particles (which lack gases and have much lower quantities of adsorbed organic compounds). The studies showed that exposure to high concentrations of either PM in whole DE or carbon black particles can cause lung tumors (Mauderly et al. 1994; Heinrich et al. 1995). These results have been widely interpreted as suggesting that the mechanisms of carcinogenesis in rats are likely to be related to exposures to high doses of the particles themselves and to possible lung overload rather than to the gases or adsorbed organic compounds (McClellan 1996; Kittelson et al. 2002; Bunn et al. 2004; Hesterberg et al. 2006).

By contrast, the studies in mice have yielded less consistent results. Some showed an increase in the incidence of lung neoplasms relative to the control animals (Pepelko and Peirano 1983; Heinrich et al. 1986; Takemoto et al. 1986), while some did not (Heinrich et al. 1995; Mauderly et al. 1996). In the study by Pepelko and Peirano (1983), Sencar mice were exposed from conception; in the study by Takemoto and colleagues (1986), ICR and C57BL mice were exposed as newborns; in the study by Heinrich and colleagues (1986) in NMRI mice, the spontaneous lung tumor rate of the control groups was unusually low compared with historical controls at the same institute (Heinrich et al. 1995). The negative studies were conducted in NMRI and C57BL mice (Heinrich et al. 1995) and in CD-1 mice (Mauderly et al. 1996). The EPA (2002a) concluded that although "earlier studies provided some evidence for tumorigenic response in diesel-exposed mice, no increases were reported in the two most recent studies (Heinrich et al. 1995; Mauderly et al. 1996) which utilized large group sizes and were well designed and conducted. Overall the results in mice must therefore be considered as unequivocal."

The two studies conducted in hamsters (Heinrich et al. 1986; Brightwell et al. 1989), using concentrations of DPM ranging from 4 to 6.6 mg/m^3 and numbers of animals similar to those in the rat studies, did not show any increases in benign or malignant lung tumors.

Several studies evaluated the carcinogenicity of filtered DE (i.e., with the same concentrations of gaseous components but without the particulate phase) in rats. All of the studies reported no increase in lung tumors in the animals exposed to filtered exhaust relative to the control animals, and all reported increases in the rats exposed to whole DE (Heinrich et al. 1986; Iwai et al. 1986, 1997; Brightwell et al. 1989). In their first study Iwai and colleagues (1986) found an increase in splenic malignant lymphomas in animals exposed to either filtered or unfiltered DE. The EPA assessment (2002a) noted that "this is the only report to date of tumor induction at an extrarespiratory site by inhaled DE in animals."

Only two studies evaluated the carcinogenicity of filtered exhaust in mice (Heinrich et al. 1986, 1995). In the 1986 study, which showed an increased incidence of lung tumors (from 13% to 32%) in the animals exposed to whole DE, a similar increase was also noted with filtered DE. However, the incidence of multifocal bronchoalveolar hyperplasia, interstitial fibrosis, and alveolar lipoproteinosis was greater in animals exposed to whole DE than in animals exposed to filtered DE. Studies conducted by the same group in the 1990s in two species did not find any significant tumor incidence after exposure to whole DE or filtered DE relative to animals exposed to clear air.

The EPA (2002a) concluded that "little direct evidence exists for carcinogenicity of the vapor phase of DE in laboratory animals at the concentrations tested."

Epidemiology

Among the more significant epidemiologic studies, only two have reported quantitative exposure data and been used for risk assessment: a series of U.S.-railroad-worker studies (Garshick et al. 1987, 1988, 2004, 2006; Woskie et al. 1988a,b; Larkin et al. 2000; Laden et al. 2006) and a U.S.-teamster study (Steenland et al. 1990, 1992; Zaebst et al. 1991). Although exposure data were collected from current workers, the studies were not designed to include the development of comprehensive exposure models nor to extrapolate from historical exposures.

The U.S. railroad industry converted from coal- to diesel-powered locomotives after World War II and during the 1950s. By 1959, 95% of the locomotives in service were diesel-powered. Garshick and colleagues (1987) conducted a case–control study of lung cancer deaths (with

data collected for 12 months in 1981 and 1982) in railroad workers who had had at least 10 years of service and were eligible for retirement benefits. There was a significant odds ratio (OR) of 1.41 (CI, 1.06–1.88) for lung-cancer death, controlling for smoking (including next-of-kin smoking history) and for asbestos exposure, among men 64 years of age or younger with 20 years of service in jobs associated with DE exposure starting in 1959. Garshick and colleagues (1988) also conducted a retrospective cohort study of workers aged 40 to 64 who were employed in 1959 in one of 39 jobs surveyed in an industrial-hygiene study (Woskie et al. 1988a,b). The cohort was followed up through 1980, and exposure was assessed as the cumulative years in jobs associated with DE exposure (assuming, again, that exposure started in 1959). The highest relative risk (RR) was observed for those workers who were 40 to 44 years of age in 1959, the group with the longest possible duration of exposure (RR =1.45; CI, 1.11–1.89). The same investigators performed additional analyses controlling for smoking, using indirect methods, of the follow-up data through 1976 (Larkin et al. 2000). The smoking-adjusted RR for lung cancer was 1.44 (CI, 1.01–2.05) compared with 1.58 (CI, 1.14–2.20) unadjusted.

In 1999, HEI critically evaluated the results of the studies of the railroad-worker cohort and determined if the data from them were adequate for quantitative risk assessment (HEI Diesel Epidemiology Expert Panel 1999). Both the HEI expert panel (1999) and an EPA consultant (Crump 1999) obtained the raw data and independently replotted the RR of lung cancer and different estimates of cumulative actual months exposure. Crump (1999) found a decreasing RR of lung cancer with cumulative exposure; the HEI panel (1999) found a similar decreasing RR with duration of employment for the main job categories (train workers, shop workers, and clerks) yet found that train workers had a higher risk than shop workers or clerks. The HEI panel concluded that "the railroad cohort study…has very limited utility for quantitative risk assessment of lifetime cancer risk from exposure to ambient levels of diesel exhaust" (HEI Diesel Epidemiology Expert Panel 1999).

The cohort was further evaluated by including deaths from 1981 to 1996 (Watanabe and Ohsawa 2002). Workers operating trains (engineers and conductors) who were between 40 and 44 years of age in 1959 had an RR of lung-cancer mortality of 1.49 (CI, 1.30–1.70). This elevated lung-cancer risk persisted after adjustment for smoking, as derived from the 1987 case–control study (Garshick et al. 2006). The risk of lung cancer, however, did not increase with increasing years of work in these jobs, confirming the findings of the earlier Crump (1999) and HEI (HEI Diesel Epidemiology Expert Panel 1999) analyses. Garshick and colleagues argued that the lack of dose–response was caused by a healthy-worker survivor effect and might also arise from exposure to coal-combustion products before 1959 and improvements in diesel-engine efficiency with reduced emissions over time. However, in a later analysis in which the years of exposure were weighted using estimates of the rate at which each railroad converted from coal to diesel (Laden et al. 2006), there was an increasing risk with increasing years of work in diesel-exposed jobs for workers who started work in 1945 or later as diesel locomotives were introduced.

The strengths of the railroad-worker studies were the very large cohort, extensive follow-up, and timing in relation to the conversion from coal to diesel; the strengths of the case–control study were ascertainment of smoking, asbestos exposure, and other potential confounders.

The National Institute of Occupational Safety and Health conducted a large case–control study of lung cancer in retired trucking-company teamsters whose deaths occurred between 1982 and 1983 (Steenland et al. 1990, 1992, 1998). An industrial-hygiene survey of PM and elemental carbon exposures in the trucking industry accompanied the epidemiologic study, thereby validating exposure assignments (Zaebst et al. 1991). The OR for lung cancer, controlling for smoking, was 1.69 (CI, 0.92–3.09) for mechanics, who had the highest exposure to elemental carbon. The ORs for short-haul (city) and long-haul (highway) drivers were 1.31 (CI, 0.81–2.11) and 1.27 (CI, 0.83–1.93), respectively (Steenland et al. 1990). The authors observed positive trends in lung-cancer risk with duration of employment for long-haul drivers after 1959, when long-haul trucks had generally converted to diesel. However, lung cancer risk was similarly elevated in short-haul drivers, whose trucks were still primarily gasoline-powered and who drove in urban settings, suggesting a contribution of traffic emissions not limited to diesel. In its 1999 review, HEI noted that "the investigators' analyses of the teamster data reported an exposure–response relationship that may be useful for quantitative risk assessment" (HEI Diesel Epidemiology Expert Panel 1999), but that further exploration of uncertainties and assumptions was needed.

Critiques of the studies of Garshick and colleagues and Steenland and colleagues for use in quantitative risk assessment cited the lack of concurrent exposure data, lack of dose–response relationship, possible misclassification of smoking habits and DE exposure, and, for the teamster study, a possibly insufficient latency period (because of uncertainties about the conversion to diesel in the trucking industry) (Hesterberg et al. 2006).

The increase in lung-cancer mortality observed in railroad workers and teamsters is consistent with findings in a large number of studies of a weak association between

work in a job associated with diesel exposure and death from lung cancer (Cohen and Higgins 1995).

Regulatory Summary

Because of the potential health effects of exposure to DE, the EPA (1999a) has included both DPM and diesel organic gases in its list of MSATs whose emissions might be further regulated. It has also completed its *Health Assessment Document for Diesel Engine Exhaust* (EPA 2002a) to "provide information about the potential for diesel engine exhaust to pose environmental health hazards." Other agencies, such as the National Institute for Occupational Safety and Health (1988), the International Agency for Research on Cancer (1989), the World Health Organization (1996), and the National Toxicology Program (2005), have reviewed the literature on the health effects of DE exposure and evaluated the human carcinogenic potential of DE. Based on the epidemiologic data and supporting evidence from animal and in vitro studies of DE, all of these agencies have classified DE as a probable human carcinogen. Based on a review of the health risk of exposure to DE conducted by the California EPA (1998), the California Air Resources Board (ARB) has designated DPM as a toxic air contaminant for which additional control measures might be needed (ARB 1999). A summary of these evaluations is shown in Table 10.

The EPA emphasized that "while EPA believes that the assessment conclusions apply to the general use of diesel engines today, as cleaner diesel engines replace a substantial number of existing engines, the general applicability of the conclusions in this health assessment document will need to be reevaluated" (Foreword by Paul Gilman, EPA 2002a).

CHRONIC NONCANCER HEALTH EFFECTS

Regulatory Summary

In addition to evaluating the potential cancer hazard associated with DE, the EPA conducted an assessment of potential chronic noncancer health effects to derive an inhalation reference concentration (RfC), an estimate of a daily inhalation exposure of the human population (including sensitive subgroups) that is "likely to be without an appreciable risk of deleterious effects during a lifetime" (EPA 2003c). For this assessment, the EPA used the information gathered in its 2002 DE health assessment. The evidence in support of the RfC was derived mainly from high-exposure long-term animal inhalation studies showing "consistent findings of inflammatory, histopathological (including fibrosis), and functional changes in the pulmonary and tracheobronchial regions of laboratory animals, including the rat, mouse, hamster, guinea pig, monkey, and cat," with some corroborative evidence from occupational studies suggesting the occurrence of mostly transient respiratory symptoms and impairment of lung function. Based on these studies, the EPA concluded that "chronic respiratory effects are the principal noncancer hazard to humans from long-term environmental exposure to DE." The RfC of 5 $\mu g/m^3$ for chronic noncancer respiratory effects of exposure to DPM was calculated from dose–response data on inflammatory and histopathological changes in the lung from chronic-inhalation studies in rats. The EPA commented that "other effects—such as neurological, growth and survival, lowered resistance to respiratory infections, liver effects—are observed in animal studies at higher concentrations than those producing the respiratory effects." The California EPA's *Health Risk Assessment of Diesel Exhaust* reached similar conclusions (California EPA 1998).

SHORT-TERM NONCANCER HEALTH EFFECTS

Inflammation and Allergic Responses

Asthma and allergies have emerged as important public health issues, and many investigations are underway to examine the broad variety of factors that might cause or exacerbate them. In this context, epidemiologic studies in adults and children have raised concerns that exposure to traffic-related air pollution (though not specifically DE) might be associated with the exacerbation of asthma and allergy symptoms (Duhme et al. 1996; English et al. 1999; Brauer et al. 2002; Janssen et al. 2003; Nicolai et al. 2003; Ryan et al. 2005). Because of these concerns, a number of studies have been conducted in which healthy and asthmatic participants have been exposed to DE or DPM to evaluate effects on allergic responses and the respiratory system. These studies showed that DE can cause some short-term effects on the airways under some experimental conditions. The results are summarized here.

Controlled exposures of healthy human participants to relatively high concentrations of fresh DE (300 $\mu g/m^3$ for 1 hour) from a 1990 diesel engine revealed modest changes in some inflammatory markers in lung sputum, lung biopsies, and blood but no changes in lung function (Salvi et al. 1999, 2000; Nordenhall et al. 2000). Similar studies in participants with mild asthma showed increases in airway reactivity, lung resistance, and markers of mild inflammation in sputum (no measures were taken in tissue biopsies or blood) (Nordenhall et al. 2001). In subsequent studies by the same research group involving exposure of healthy and asthmatic participants to DE containing 100 $\mu g/m^3$ DPM for 2 hours, both healthy and asthmatic participants

Table 10. Summary of Diesel Hazard Assessments[a]

Agency and Year	Findings
National Institute of Occupational Safety and Health 1988	Animal evidence "confirmatory" for carcinogenesis Human evidence "limited" DE classified as "potential occupational carcinogen" No quantitative risk assessment
International Agency for Research on Cancer 1989	"Sufficient evidence" for carcinogenicity in experimental animals Epidemiology data provide "limited evidence" for carcinogenicity DE considered a "probable" human carcinogen. No quantitative risk assessment
World Health Organization 1996	Rat data support carcinogenicity Human epidemiology data suggest "probably carcinogenic" Epidemiology studies considered "inadequate for a quantitative estimate of human risk" Rat data used for quantitative risk assessment
California Environmental Protection Agency 1998	Rat data "have demonstrated" carcinogenicity of DPM Causal association of DE and lung cancer in epidemiology studies is a "reasonable and likely explanation" Human epidemiology data used for quantitative risk assessment because of uncertainties in extrapolation from animals to humans DPM designated a "toxic air contaminant" (by California Air Resources Board)
National Toxicology Program 2005	DPM listed as "reasonably anticipated to be a human carcinogen" based on findings of elevated lung cancer in occupational groups exposed to DE and supporting animal and mechanistic studies No quantitative risk assessment
Environmental Protection Agency 2002a	Diesel emissions considered "likely to be carcinogenic to humans" No quantitative risk assessment Perspective of the range of possible lung-cancer risk was developed on the basis of occupational epidemiologic studies Evidence considered: • Strong but less-than-sufficient epidemiologic evidence • Rat lung tumor response (occurring only at high doses that cause inhibition of particle clearance, resulting in lung particle overload) not considered relevant to effects in humans exposed to low ambient concentrations • Results in mice considered equivocal; hamster results considered negative • Evidence of carcinogenicity of DPM in rats and mice when exposed by non-inhalation routes • Extensive supportive data include mutagenic or chromosomal effects of DE and its organic constituents

[a] Abbreviations: DE = diesel exhaust; DPM = diesel-exhaust particles.

showed a small increase in airway resistance, but most changes in inflammatory parameters in bronchial alveolar lavage (BAL) or lung tissue were observed only in healthy participants (Holgate et al. 2003). Participants with asthma, however, had higher baseline inflammation than healthy participants (Holgate et al. 2003). A separate study at the same DE concentration reported an increase in subjective symptoms and mild bronchoconstriction in healthy exercising participants but no changes in airway inflammation (Mudway et al. 2004). Overall, these studies suggested that the responses of healthy and asthmatic participants to DE are variable and that the baseline level of inflammation might influence the outcome.

The question of whether DE increases the specific allergic response to an allergen, which involves different inflammatory mediators from those involved in a nonspecific inflammatory response, has been addressed experimentally using an allergen challenge combined with exposure to DPM. Healthy and asthmatic volunteers were exposed to 300 µg of resuspended DPM (collected from

emissions generated by a 1980 diesel car engine and archived) via nasal spray (a physiologically nonrelevant method of exposure). Both groups of volunteers had enhanced production of total immunoglobulin E (IgE) in the nose (Diaz-Sanchez et al. 1994) and increased production of cytokines characteristic of both the T_H1 and T_H2 subsets of CD4+ T lymphocytes in the nasal mucosa (Diaz-Sanchez et al. 1996). These two subtypes of cells play distinct roles in the immune response. T_H1 cytokines are involved in cell-mediated immunity; T_H2 cytokines trigger IgE production by B lymphocytes and recruitment of eosinophils, which are indicators of allergic asthma. Intranasal challenge with DPM and ragweed allergen in human participants who were allergic to ragweed markedly enhanced the production of ragweed-specific IgE, but not total IgE, compared with a challenge with ragweed allergen alone, and resulted in decreased production of T_H1 cytokines and increased production of T_H2 and other cytokines (such as interleukin-6 (IL-6) (Diaz-Sanchez et al. 1997). However, these studies focused only on the nasal response and involved a bolus exposure to resuspended DPM, which might have different physicochemical properties from fresh aerosolized DPM.

Studies of allergic response in mice and rats (generally sensitized and challenged with ovalbumin as the allergen) have used different protocols for administering the allergen, different timing of the diesel exposure relative to the administration of the allergen, and different route of exposure. Because of these, the results obtained are not entirely consistent. Several studies have found evidence of adjuvant effects of DPM or DE on the production of allergen-specific IgE antibodies and inflammation (Takafuji et al. 1987; Fujimaki et al. 1997; Takano et al. 1997; Steerenberg et al. 2003; Dong et al. 2005). Steerenberg and colleagues (2003) found that the effect of DPM on ovalbumin-specific IgE production was dependent on the timing of the exposure relative to the timing of the ovalbumin administration. Some studies reported an effect of DPM on allergen-induced airway responsiveness (Hao et al. 2003; Dong et al. 2005; Matsumoto et al. 2006). In the Hao and colleagues study, which also measured ovalbumin-specific IgE production, there was no adjuvant effect of DPM.

Taken together, human and animal studies suggest that, under certain experimental conditions, DPM (from older engines) might induce markers of nonspecific inflammation in healthy and asthmatic participants, might cause changes in respiratory function, and might act as an adjuvant to increase the specific immune response to an allergen. Findings of adjuvant effects in humans, however, need to be validated in studies with more relevant exposure conditions. Also, there is some evidence in animals that other particles, such as Kanto loam dust, fly ash, carbon black, and alum (Maejima et al. 1997) or residual-oil fly ash (Lambert et al. 1999), can induce similar responses, raising the question of whether this is a DE-specific response or one induced by exposure to particles in general. However, conclusions about the studies in animals are difficult to generalize to humans because different effects can be observed when different animal species or study protocols are used.

Regulatory Summary

The EPA (2002a) concluded that "as with humans, there are animal data suggesting that DEP [DPM] is a possible factor in the increasing incidence of allergic hypersensitivity." The EPA concluded that "additional research is needed to further characterize the immunological effects of DE and to determine whether or not the immunological effects constitute a low-exposure hazard." In its determination of the inhalation RfC, the EPA (2003c) commented that the human and animal data for the immunological effects of DPM exposure (i.e., exacerbation of allergenicity, and asthma symptoms) are currently inadequate for dose–response evaluation and these data did not support further adjustment of the RfC. The California EPA *Health Risk Assessment* (1998) also concluded that the available information could not be used to develop quantitative estimates for determining the RfC but noted that "the potential relevance of these immunological endpoints to public health is very high, due to reports of large numbers of individuals with respiratory allergies and asthma in urban areas."

EFFECTS ON RESPIRATORY INFECTIONS

Animal Studies

A few studies have been conducted in mice and rats to evaluate the effects of inhaled DE on host defense against bacterial or viral agents. In these studies, mice or rats were exposed by inhalation to DE with high concentrations of DPM (generally greater than 2 mg/m^3; one study used 20 mg/m^3) or to clean air for 5 days or longer and then infected with a respiratory bacterium (*Listeria*) or virus (influenza) (Hahon et al. 1985; Castranova et al. 2001; Yin et al. 2004). The studies found increased lung injury, inflammatory response, and bacterial or viral loads in the animals exposed to DE compared with the animals exposed to clean air. Harrod and colleagues (2003) exposed mice to a high (1 mg/m^3) or low (0.03 mg/m^3) concentration of DPM, followed by infection with respiratory syncytial virus. They found a dose-related increase in the expression of viral mRNA, inflammatory mediators, and

interferon levels (a response that was the opposite of that reported in the Yin et al. [2004] study using exposures of 20 mg/m^3 DPM). Other changes included a dose-related increase in mucus-cell metaplasia and lung inflammation. A more recent study demonstrated that the use of low-sulfur fuel and a catalyzed trap completely, or almost completely, eliminated lung inflammation, oxidative stress, and the responses to viral infection induced after exposure to whole DE, compared with values observed in this and previous studies at the same exhaust dilution (McDonald et al. 2004). Overall, these studies suggested that DE might lower resistance to respiratory infections under certain conditions; these results have yet to be replicated.

Regulatory Summary

Based on two early studies in which mice were exposed to 2 to 8 mg/m^3 DPM (Campbell et al. 1981; Hahon et al. 1985), the EPA concluded that "exposure to DPM can reduce an animal's resistance to respiratory infection" but noted that these effects were observed only at very high concentrations (EPA 2002a). The California EPA *Health Risk Assessment* (1998) also stated that "inhalation or direct application of diesel into the respiratory tract of animals…increased susceptibility of exposed animals to lung infections." Because these effects were observed at exposure concentrations higher than those associated with other effects (such as lung inflammation and histopathological changes), the reduced response to respiratory infections was not used by the EPA in its determination of the RfC for DE.

EFFECTS ON REPRODUCTIVE FUNCTION AND FETAL DEVELOPMENT

Animal Studies

Although there is no evidence that maternal exposure to DE is associated with fetal malformation (EPA 2002a; Tsukue et al. 2002), some studies using a variety of exposure protocols and animal models and examining different endpoints suggested that exposure of pregnant rodents might cause subtle changes in both the mothers and their offspring. Maternal changes included alterations of hormonal balance after exposure during pregnancy to DE with 5.6 mg/m^3 DPM (Watanabe and Kurita 2001) and, after giving birth, a lower rate of nest construction after exposure during pregnancy to a DPM concentration of 3 mg/m^3 (though not at lower concentrations) (Tsukue et al. 2002). The effects reported in offspring exposed in utero included delayed or disturbed differentiation of the testis, ovary, and thymus (Watanabe and Kurita 2001) and delayed gonadal maturation and lower weight gain in the group exposed in utero to 3 mg/m^3 (Tsukue et al. 2002).

Some studies appear to suggest effects on the male reproductive system. Changes in endocrine function, decreased sperm production, and changes in hyaluronidase activity in growing male rats exposed from birth to 3 months of age to DE at a concentration of 5.6 mg/m^3 were also reported (Watanabe and Oonuki 1999). However, a study in which male rats were exposed to DE at concentrations of 0.3, 1.0, or 3.0 mg/m^3 for 8 months beginning at 6 weeks of age did not show changes in sperm counts (Tsukue et al. 2001, 2002). Possible reasons for the difference between the findings of this study and the one by Watanabe and Oonuki (1999) were the higher DPM concentrations and the younger age of the animals in the latter study. In both studies, the authors observed increased levels of serum testosterone. Mice seemed to be more sensitive than rats, based on a study by Yoshida and colleagues (1999), who reported a dose-dependent decrease in daily sperm production in mice exposed to DE at DPM concentrations of 0.3, 1.0, or 3.0 mg/m^3. No effects on the weight of the testis, epididymis, or adrenal glands were observed. Ultrastructural changes in Leydig cells (which are involved in spermatogenesis) were observed even at the lowest DPM concentration.

A recent study evaluated the effect of in utero exposure to whole DE on sperm counts and Sertoli cells in the adults rats. Pregnant mice were exposed to DE at 0.17 or 1.7 mg/m^3 DPM concentrations or filtered DE with similar levels of NO_2 (Watanabe 2005). The author reported that there were fewer Sertoli cells and sperm cells in the rats exposed in utero to both high and low concentrations of whole DE or filtered DE. Daily sperm production was also decreased, but no dose–response relationship was observed for any of these effects.

Regulatory Summary

The EPA (2002a) concluded that "DE is not likely to pose reproductive or developmental hazard to humans." The agency (2002a) also noted that "no teratogenic, embryotoxic, fetotoxic, or female reproductive effects were observed in mice, rats, or rabbits at exposure levels up to 12 mg/m^3 DPM." Although "effects on sperm morphology and number were reported in hamsters and mice exposed to high levels of DPM…no adverse effects were observed in sperm obtained from monkeys exposed at 12 mg/m^3 for 7 hours/day, 5 days/week for 104 weeks" (EPA 2002a). The California EPA *Health Risk Assessment* (1998) concluded that "the available literature does not provide sufficient information to determine whether or not diesel exhaust exposure induces reproductive, developmental, or teratogenic effects in humans." Because a number of studies have been published since the EPA and ARB assessments, this evaluation might need to be updated.

EFFECTS ON THE CARDIOVASCULAR SYSTEM

Animal and Human Studies

DPM contributes to the mixture of PM in ambient air, especially in urban environments, and it is likely that it contributes to some of the health effects associated with ambient PM. Epidemiologic studies in many places have shown that there is an association between short-term increases in PM concentrations and mortality and morbidity (and that people with cardiovascular or pulmonary disease seem to be most susceptible; see Pope and Dockery 2006 for a recent review).

Recent findings suggest some plausible mechanisms for how exposure to low concentrations of ambient or laboratory-generated PM might initiate a sequence of events that affect the cardiovascular or pulmonary systems: (1) induction of oxidative stress and inflammatory responses in the airways (such as increases in neutrophil number and levels of cytokines and chemokines); (2) induction of systemic inflammatory and other vascular responses (e.g., changes in blood pressure; levels of fibrinogen, C-reactive protein, and endothelins; plasma viscosity; and platelet numbers—several of which are associated with the risk of cardiovascular disease); and (3) dysfunction of the autonomic nervous system, leading to cardiac electrophysiologic changes and possibly to cardiac events, such as myocardial infarction or arrhythmias (Brook et al. 2004). Some of these effects have been observed, although not consistently, in experimental studies using a variety of animal models and types of PM (including concentrated ambient PM; laboratory-generated PM, such as metal-oxide PM; and source-specific PM, such as diesel, coal fly ash, and residual-oil fly ash).

Because DPM has some characteristics that have been hypothesized to be potentially associated with cardiovascular changes, including small particles with high surface area and adsorbed metals and organic components, some studies have been conducted recently to begin to evaluate the effects of DPM on the cardiovascular system. DE also contains a number of oxidant gases that are irritants and can cause oxidative stress.

One study in animals has shown that exposure of healthy F344 rats to DE for 6 months at 1 mg/m^3 PM (6 hours/day, 7 days/week) caused a small decrease in blood coagulation factor VII in both males (12%) and females (27%) (Reed et al. 2004). Spontaneously hypertensive rats exposed to the same concentration of DPM for 1 week had elevated heart rates throughout the exposure and significantly prolonged PQ intervals in their electrocardiograms, which might indicate a risk of arrhythmia (Campen et al. 2003). More recently, the same group of investigators reported that both the gaseous components of DE (i.e., filtered exhaust) and whole exhaust (with 3 mg/m^3 DPM) induced a decrease in heart rate and electrocardiogram changes consistent with myocardial ischemia in a mouse strain that spontaneously develops atherosclerosis (Campen et al. 2005). Whole DE induced airway inflammation, and filtered exhaust did not, suggesting different roles for the particulate and gaseous exhaust components. Recently, Mills and colleagues (2005) reported that exposure of human volunteers to DE (with 300 µg/m^3 DPM for 1 hour) impaired the regulation of vascular tone and fibrinolysis. These changes might be involved in the pathway to thrombosis and myocardial infarction. The studies appeared to suggest effects of DE on the cardiovascular system that are consistent with those attributed to PM as a whole.

Regulatory Summary

Because of a lack of data at the time, neither the EPA nor the California EPA considered these outcomes in their assessments.

SUMMARY

The results of studies investigating exposure to DE from older and more current engines indicate effects on the respiratory, reproductive, and cardiovascular systems. Extrapolation of these findings to people exposed to much lower concentrations of DE components than those used in experimental studies or in epidemiologic studies of occupationally exposed workers can be challenging.

Despite these challenges, many agencies have determined that DE is of sufficient concern to merit action to reduce emissions. New diesel engines with control systems meeting 2007 emission standards for heavy-duty on-highway vehicles are now on the market. Emissions from four such engines will be characterized in detail in the Advanced Collaborative Emissions Study (ACES), which is a joint effort of the Coordinating Research Council and HEI; chronic and acute health endpoints will be assessed for one of the engines. Although durable older engines with higher emissions will continue to be used, these new engines, and those designed to meet the more stringent 2010 standards, will gradually become more common, with substantial replacement expected by 2030.

DIESEL EXHAUST REFERENCES

Brauer M, Hoek G, Van Vliet P, Meliefste K, Fischer PH, Wijga A, Koopman LP, Neijens HJ, Gerritsen J. Kerkhof M, Heinrich J, Bellander T, Brunekreef B. 2002. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. Am J Respir Crit Care Med 166(8):1092–1098.

Brightwell J, Fouillet X, Cassano-Zoppi AL, Bernstein D, Crawley F, Duchosal F, Gatz R, Perczel S, Pfeifer H. 1989. Tumours of the respiratory tract in rats and hamsters following chronic inhalation of engine exhaust emissions. J Appl Toxicol 9(1):23–31.

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R, Mittleman M, Samet J, Smith SC Jr, Tager I. 2004. Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation 109:2655–71.

Bunn WB 3rd, Hesterberg TW, Valberg PA, Slavin TJ, Hart G, Lapin CA. 2004. A reevaluation of the literature regarding the health assessment of diesel engine exhaust. Inhal Toxicol 16:889–900.

California Air Resources Board. 1999. Toxic Air Contaminants (TAC) Identification List. Substance by Category (last updated December 1999). Available from *www.arb.ca.gov/toxics/cattable.htm.* Accessed October 18, 2002; March 2007.

California Environmental Protection Agency. 1998. Part B: Health Risk Assessment for Diesel Exhaust. In: Proposed Identification of Diesel Exhaust as an Air Toxic Contaminant. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available from *ftp://ftp.arb.ca.gov/carbis/regact/diesltac/partb.pdf.*

California Environmental Protection Agency. 2003. Health Effects of Diesel Exhaust. Office of Environmental Health Hazard Assessment, Sacramento, CA. Available from *www.oehha.ca.gov/public_info/facts/dieselfacts.html.*

Campbell KI, George EL, Washington IS Jr. 1981. Enhanced susceptibility to infection in mice after exposure to dilute exhaust from light duty diesel engines. Environ Int 5(4-6): 377–382.

Campen MJ, McDonald JD, Gigliotti AP, Seilkop SK, Reed MD, Benson JM. 2003. Cardiovascular effects of inhaled diesel exhaust in spontaneously hypertensive rats. Cardiovasc Toxicol 3(4):353–61.

Campen MJ, Babu NS, Helms GA, Pett S, Wernly J, Mehran R, McDonald JD. 2005. Nonparticulate components of diesel exhaust promote constriction in coronary arteries from ApoE-/- mice. Toxicol Sci 88(1):95–102.

Carslaw D, Beevers SD. 2004. New Directions: Should road vehicle emissions legislation consider primary NO_2? Atmos Environ 38:1233–1234.

Castranova V, Ma JY, Yang HM, Antonini JM, Butterworth L, Barger MW, Roberts J, Ma JK. 2001. Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. Environ Health Perspect 109 Suppl 4:609–12.

Cohen A, Higgins MWP. 1995. Health Effects of Diesel Exhaust: Epidemiology. In: Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects (HEI Diesel Working Group, ed.). Health Effects Institute, Cambridge, MA.

Crump KS. 1999. Lung cancer mortality and diesel exhaust: Reanalysis of a retrospective cohort study of U.S. railroad workers. Inhalation Toxicol 11(1):1–17.

Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A. 1994. Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. J Clin Invest 94(4):1417–25.

Diaz-Sanchez D, Tsien A, Casillas A, Dotson AR, Saxon A. 1996. Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. J Allergy Clin Immunol 98(1):114–23.

Diaz-Sanchez D, Tsien A, Fleming J, Saxon A. 1997. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol 158(5):2406–13.

Dong CC, Yin XJ, Ma JY, Millecchia L, Wu ZX, Barger MW, Roberts JR, Antonini JM, Dey RD, Ma JK. 2005. Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. Toxicol Sci 88:202–212.

Duhme H, Weiland SK, Keil U, Kraemer B, Schmid M, Stender M, Chambless L. 1996. The association between self-reported symptoms of asthma and allergic rhinitis and self-reported traffic density on street of residence in adolescents. Epidemiology 7(6):578–582.

English P, Neutra R, Scalf R, Sullivan M, Waller L, Zhu L. 1999. Examining associations between childhood asthma

and traffic flow using a geographic information system. Environ Health Perspect 107(9):761–767.

Environmental Protection Agency (U.S.). 1998b. Control of Emissions of Air Pollution from Nonroad Diesel Engines; Final Rule. 40 CFR Parts 9, 86, and 89. Fed Regist 63: 56967–57023.

Environmental Protection Agency (U.S.). 1998c. Emission Standards for Locomotives and Locomotive Engines; Final Rule. 40 CFR Parts 85, 89, and 92. Fed Regist 63:18977–19026.

Environmental Protection Agency (U.S.). 1999a. National Air Toxics Program: The Integrated Urban Air Toxics Strategy. 40 CFR Parts 80 and 86: Federal Register 64: 38706-38740.

Environmental Protection Agency (U.S.). 2000a. Control of Air Pollution from New Motor Vehicles: Tier 2 Motor Vehicle Emissions Standards and Gasoline Sulfur Control Requirements; Final Rule. 40 CFR Parts 80, 85, and 86. Fed Regist 65:6747–6796.

Environmental Protection Agency (U.S.). 2001a. Control of Air Pollution from New Motor Vehicles: Heavy-Duty Engine and Vehicle Emissions Standards and Highway Diesel Fuel Sulfur Control Requirements; Final Rule. 40 CFR Parts 69, 80, and 86. Fed Regist 66:5002–5193.

Environmental Protection Agency (U.S.). 2002a. Health Assessment Document for Diesel Engine Exhaust. EPA/600/8-90/057F. NTIS PB 2002-107661. National Center for Environmental Assessment, Washington, DC. Available from *http://cfpub2.epa.gov/ncea/*.

Environmental Protection Agency (U.S.). 2003c. Integrated Risk Information System: Diesel Engine Exhaust (last updated February 28, 2003). Available from U.S. Environmental Protection Agency, *www.epa.gov/iris/subst/0642.htm.* Accessed July 2, 2004; April 2007.

Environmental Protection Agency (U.S.). 2004b. Control of Emissions of Air Pollution from Nonroad Diesel Engines and Fuel; Final Rule. 40 CFR Parts 9, 69, 80, 86, 94, 1039, 1048, 1051, 1065, and 1068. Fed Regist 69:38957–39273.

Fujimaki H, Saneyoshi K, Shiraishi F, Imai T, Endo T. 1997. Inhalation of diesel exhaust enhances antigen-specific IgE antibody production in mice. Toxicology 116:227–233.

Garshick E, Laden F, Hart JE, Rosner B, Smith TJ, Dockery DW, Speizer FE. 2004. Lung cancer in railroad workers exposed to diesel exhaust. Environ Health Perspect 112(15): 1539–43.

Garshick E, Laden F, Hart JE, Smith TJ, Rosner B. 2006. Smoking imputation and lung cancer in railroad workers exposed to diesel exhaust. Am J Ind Med 49(9):709–18.

Garshick E, Schenker MB, Munoz A, Segal M, Smith TJ, Woskie SR, Hammond SK, Speizer FE. 1987. A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am Rev Respir Dis 135(6):1242–8.

Garshick E, Schenker MB, Munoz A, Segal M, Smith TJ, Woskie SR, Hammond SK, Speizer FE. 1988. A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. Am Rev Respir Dis 137(4):820–5.

Gertler AW, Gillies JA, Pierson WR, Rogers CF, Sagebiel JC, Abu-Allaban M, Coulombe W, Tarnay L, Cahill TA. 2002. Real-world particulate matter and gaseous emissions from motor vehicles in a highway tunnel. In: Emissions from Diesel and Gasoline Engines Measured in Highway Tunnels. Research Report 107. Health Effects Institute, Boston, MA.

Hahon N, Booth JA, Green F, Lewis TR. 1985. Influenza virus infection in mice after exposure to coal dust and diesel engine emissions. Environ Res 37(1):44–60.

Hao M, Comier S, Wang M, Lee JJ, Nel A. 2003. Diesel exhaust particles exert acute effects on airway inflammation and function in murine allergen provocation models. J Allergy Clin Immunol 112:905–914.

Harrod KS, Jaramillo RJ, Rosenberger CL, Wang SZ, Berger JA, McDonald JD, Reed MD. 2003. Increased susceptibility to RSV infection by exposure to inhaled diesel engine emissions. Am J Respir Cell Mol Biol 28(4):451–63.

Health Effects Institute Diesel Epidemiology Expert Panel. 1999. Diesel Emissions and Lung Cancer: Epidemiology and Quantitative Risk Assessment. A Special Report. Health Effects Institute, Cambridge, MA.

Health Effects Institute Diesel Working Group. 1995. Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects. Special Report. Health Effects Institute, Cambridge, MA.

Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levsen K. 1995. Chronic inhalation exposure of Wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal Toxicol 7(4):533–556.

Heinrich U, Muhle H, Takenaka S, Ernst H, Fuhst R, Mohr U, Pott F, Stober W. 1986. Chronic effects on the respiratory tract of hamsters, mice and rats after long-term inhalation of

high concentrations of filtered and unfiltered diesel engine emissions. J Appl Toxicol 6(6):383–95.

Hemminki K, Zhang LF, Kruger J, Autrup H, Tornqvist M, Norbeck HE. 1994. Exposure of bus and taxi drivers to urban air pollutants as measured by DNA and protein adducts. Toxicol Lett 72(1-3):171–4.

Hesterberg TW, Bunn WB 3rd, Chase GR, Valberg PA, Slavin TJ, Lapin CA, Hart GA. 2006. A critical assessment of studies on the carcinogenic potential of diesel exhaust. Crit Rev Toxicol 36(9):727–76.

Holgate ST, Sandström T, Frew AJ, Stenfors N, Nördenhall C, Salvi S, Blomberg A, Helleday R, Södenberg M. 2003. Part I: Healthy and asthmatic subjects exposed to diesel exhaust. In: Health Effects of Acute Exposure to Air Pollution. Research Report 112. Health Effects Institute, Boston, MA.

Hou SM, Lambert B, Hemminki K. 1995. Relationship between hprt mutant frequency, aromatic DNA adducts and genotypes for GSTM1 and NAT2 in bus maintenance workers. Carcinogenesis 16(8):1913–7.

International Agency for Research on Cancer. 1989. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. IARC Monographs of the Evaluation of Carcinogenic Risks to Humans. Volume 46. World Health Organization, Lyon, France. Summary available from *http://monographs.iarc.fr/ENG/Monographs/vol49/volume49.pdf.*

Ishinishi N, Kuwabara N, Nagase S, Suzuki T, Ishiwata S, Kohno T. 1986. Long-term inhalation studies on effects of exhaust from heavy and light duty diesel engines on F344 rats. Dev Toxicol Environ Sci 13:329–348.

Iwai K, Higuchi K, Udagawa T, Ohtomo K, Kawabata Y. 1997. Lung tumor induced by long-term inhalation or intratracheal instillation of diesel exhaust particles. Exp Toxicol Pathol 49(5):393–401.

Iwai K, Udagawa T, Yamagishi M, Yamada H. 1986. Long-term inhalation studies of diesel exhaust on F344 SPF rats. Incidence of lung cancer and lymphoma. Dev Toxicol Environ Sci 13:349–360.

Janssen NAH, Brunekreef B, Van Vliet P, Aarts F, Meliefste K, Harssema H, Fischer P. 2003. The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. Environ Health Perspect 111(12): 1512–1518.

Kittelson D, Watts W, Johnson J. 2002. Diesel Aerosol Sampling Methodology: CRC E-43 Final Report. CRC E-43. Coordinating Research Council, Alpharetta, GA.

Kittelson DB, Watts WF, Johnson JP, Rowntree CJ, Goodier SP, Payne MJ, Goodier S, Warrens C, Preston WH, Zink U, Ortiz M, Goersmann C, Twigg MV, Walker AP, Caldow R. 2006. On-road evaluation of two diesel exhaust aftertreatment devices. J Aerosol Sci 37:1140–1151.

Laden F, Hart JE, Eschenroeder A, Smith TJ, Garshick E. 2006. Historical estimation of diesel exhaust exposure in a cohort study of U.S. railroad workers and lung cancer. Cancer Causes Control 17(7):911–9.

Lambert AL, Dong W, Winsett DW, Selgrade MK, Gilmour MI. 1999. Residual oil fly ash exposure enhances allergic sensitization to house dust mite. Toxicol Appl Pharmacol 158(3):269–77.

Lanni T. 2003. Fine urban and precursor emissions control for diesel urban transit buses. Environ Pollut 123:427–37.

Larkin EK, Smith TJ, Stayner L, Rosner B, Speizer FE, Garshick E. 2000. Diesel exhaust exposure and lung cancer: adjustment for the effect of smoking in a retrospective cohort study. Am J Ind Med 38(4):399–409.

LeTavec C, Uihlein J, Vertin K, Chatterjee S, Wayne S, Clark N, Gautam M, Thompson G, Lyons D, Hallstrom K, Chandler K, Coburn T. 2002. Year-Long Evaluation of Trucks and Buses Equipped with Passive Diesel Particulate Filters. SAE Technical Series No. 2002-01-0433. SAE International, Warrandale, PA.

Maejima K, Tamura K, Taniguchi Y, Nagase S, Tanaka H. 1997. Comparison of the effects of various fine particles on IgE antibody production in mice inhaling Japanese cedar pollen allergens. J Toxicol Environ Health 52(3):231–48.

Matsumoto A, Hiramatsu K, Li Y, Azuma A, Kudoh S, Takizawa H, Sugawara I. 2006. Repeated exposure to low-dose diesel exhaust after allergen challenge exaggerates asthmatic responses in mice. Clin Immunol 121:227–235.

Mauderly JL, Banas DA, Griffith WC, Hahn FF, Henderson RF, McClellan RO. 1996. Diesel exhaust is not a pulmonary carcinogen in CD-1 mice exposed under conditions carcinogenic to F344 rats. Fundam Appl Toxicol 30(2): 233–42.

Mauderly JL, Jones RK, Griffith WC, Henderson RF, McClellan RO. 1987. Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam Appl Toxicol 9(2):208–21.

Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang I-Y, Cheng YS, Gillett NA, Griffith WC, Henderson RF, Mitchell CE, Nikula KJ, Thomassen DG. 1994. Part I: Neoplastic and nonneoplastic lung lesions. In: Pulmonary Toxicity of Inhaled Diesel Exhaust and Carbon

Black in Chronically Exposed Rats. Research Report 68. Health Effects Institute, Cambridge, MA.

McClellan RO. 1996. Lung Cancer in Rats from Prolongued Exposure to High Concentrations of Carbonaceous Particles: Implications for Human Assessment. Inhal Toxicol 8 (Suppl):193–226.

McDonald JD, Harrod KS, Seagrave J, Seilkop SK, Mauderly JL. 2004. Effects of low sulfur fuel and a catalyzed particle trap on the composition and toxicity of diesel emissions. Environ Health Perspect 112(13):1307–12.

Mills NL, Tornqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, Boon NA, Donaldson K, Blomberg A, Sandström T, Newby DE. 2005. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112(25):3930–6.

Mudway IS, Stenfors N, Duggan ST, Roxborough H, Zielinski H, Marklund SL, Blomberg A, Frew AJ, Sandström T, Kelly FJ. 2004. An in vitro and in vivo investigation of the effects of diesel exhaust on human airway lining fluid antioxidants. Arch Biochem Biophys 423(1):200–12.

National Institute for Occupational Safety and Health (U.S.). 1988. Carcinogenic Effects of Exposure to Diesel Exhaust. NIOSH Current Intelligence Bulletin 50. Centers for Disease Control, Atlanta, GA.

National Toxicology Program (U.S.). 2005. Report on Carcinogens, Eleventh Edition. U.S. Department of Health and Human Services, Research Triangle Park, NC. Available from *http://ntp.niehs.nih.gov*.

Nicolai T, Carr D, Weiland SK, Duhme H, von Ehrenstein O, Wagner C, von Mutius E. 2003. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. Eur Respir J 21(6):956–963.

Nördenhall C, Pourazar J, Blomberg A, Levin JO, Sandström T, Adelroth E. 2000. Airway inflammation following exposure to diesel exhaust: a study of time kinetics using induced sputum. Eur Respir J 15(6):1046–51.

Nördenhall C, Pourazar J, Ledin MC, Levin JO, Sandström T, Adelroth E. 2001. Diesel exhaust enhances airway responsiveness in asthmatic subjects. Eur Respir J 17(5):909–15.

Pepelko WE, Peirano WB. 1983. Health effects of exposure to diesel engine emissions: A summary of animal studies conducted by the U.S. Environmental Protection Agency's health effects research laboratories at Cincinnati, Ohio. J Am Coll Toxicol 2:253-306.

Pope CA 3rd, Dockery DW. 2006. Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 56:709–42.

Reed MD, Gigliotti AP, McDonald JD, Seagrave JC, Seilkop SK, Mauderly JL. 2004. Health effects of subchronic exposure to environmental levels of diesel exhaust. Inhal Toxicol 16(4):177–93.

Ryan PH, Lemasters G, Biagini J, Bernstein D, Grinshpun SA, Shukla R, Wilson K, Villareal M, Burkle J, Lockey J. 2005. Is it traffic type, volume, or distance? Wheezing in infants living near truck and bus traffic. J Allergy Clin Immunol 116(2):279–284.

Sakurai H, Park K, McMurry PH, Zarling DD, Kittelson DB, Ziemann PJ. 2003. Size-dependent mixing characteristics of volatile and nonvolatile components in diesel exhaust aerosols. Environ Sci Technol 37(24):5487–95.

Salvi S, Blomberg A, Rudell B, Kelly F, Sandström T, Holgate ST, Frew A. 1999. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am J Respir Crit Care Med 159(3):702–9.

Salvi SS, Nördenhall C, Blomberg A, Rudell B, Pourazar J, Kelly FJ, Wilson S, Sandström T, Holgate ST, Frew AJ. 2000. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. Am J Respir Crit Care Med 161(2 Pt 1):550–7.

Shirnamé-Moré L. 1995. Genotoxicity of diesel emissions Part I: Mutagenicity and other genetic effects. In: Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects (HEI Diesel Working Group). Health Effects Institute, Cambridge, MA.

Steenland K, Deddens J, Stayner L. 1998. Diesel exhaust and lung cancer in the trucking industry: exposure-response analyses and risk assessment. Am J Ind Med 34(3):220–8.

Steenland K, Silverman D, Zaebst D. 1992. Exposure to diesel exhaust in the trucking industry and possible relationships with lung cancer. Am J Ind Med 21(6):887–90.

Steenland NK, Silverman DT, Hornung RW. 1990. Case-control study of lung cancer and truck driving in the Teamsters Union. Am J Public Health 80(6):670–4.

Steerenberg PA, Withagen CE, Dormans JA, van Dalen WJ, van Loveren H, Casee FR. 2003. Adjuvant activity of various diesel exhaust and ambient particles in two allergic models. J Toxicol Environ Health A 66:1421–1439.

Takafuji S, Suzuki S, Koizumi K, Tadokoro K, Miyamoto T, Ikemori R, Muranaka M. 1987. Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. J Allergy Clin Immunol 79(4):639–645.

Takano H, Yoshikawa T, Ichinose T, Miyabara Y, Imaoka K, Sagai M. 1997. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. Am J Respir Crit Care Med 156:36–42.

Takemoto K, Yoshimura H, Katayama H. 1986. Effects of chronic inhalation exposure to diesel exhaust on the development of lung tumors in di-isopropanol-nitrosamine-treated F344 rats and newborn C57BL and ICR mice. Dev Toxicol Environ Sci 13:311–327.

Tsukue N, Toda N, Tsubone H, Sagai M, Jin WZ, Watanabe G, Taya K, Birumachi J, Suzuki AK. 2001. Diesel exhaust (DE) affects the regulation of testicular function in male Fischer 344 rats. J Toxicol Environ Health A 63(2):115–26.

Tsukue N, Tsubone H, Suzuki AK. 2002. Diesel exhaust affects the abnormal delivery in pregnant mice and the growth of their young. Inhal Toxicol 14(6):635–51.

U.S. Congress, House of Representatives. 1977. Clean Air Act Amendments of 1977. U.S. Government Printing Office, Washington, DC.

Vaaraslahti K, Virtanen A, Ristimaki J, Keskinen J. 2004. Nucleation mode formation in heavy-duty diesel exhaust with and without a particulate filter. Environ Sci Technol 38:4884-90.

Watanabe N. 2005. Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. Toxicol Lett 155(1):51–8.

Watanabe N, Kurita M. 2001. The masculinization of the fetus during pregnancy due to inhalation of diesel exhaust. Environ Health Perspect 109(2):111–9.

Watanabe N, Ohsawa M. 2002. Elevated serum immunoglobulin E to Cryptomeria japonica pollen in rats exposed to diesel exhaust during fetal and neonatal periods. BMC Pregnancy Childbirth 2:2.

Watanabe N, Oonuki Y. 1999. Inhalation of diesel engine exhaust affects spermatogenesis in growing male rats. Environ Health Perspect 107(7):539–44.

World Health Organization. 1996. Diesel Fuel and Exhaust Emissions: Environmental Health Criteria. No. 171. WHO. Geneva, Switzerland.

Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. 1988a. Estimation of the diesel exhaust exposures of railroad workers: I. Current exposures. Am J Ind Med 13:381–394.

Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. 1988b. Estimation of the diesel exhaust exposures of railroad workers: II. National and historical exposures. Am J Ind Med 13:395–404.

Yin XJ, Dong CC, Ma JY, Antonini JM, Roberts JR, Stanley CF, Schafer R, Ma JK. 2004. Suppression of cell-mediated immune responses to listeria infection by repeated exposure to diesel exhaust particles in brown Norway rats. Toxicol Sci 77(2):263–271.

Yoshida S, Sagai M, Oshio S, Umeda T, Ihara T, Sugamata M, Sugawara I, Takeda K. 1999. Exposure to diesel exhaust affects the male reproductive system of mice. Int J Androl 22:307–315.

Zaebst DD, Clapp DE, Blade LM, Marlow DA, Steenland K, Hornung RW, Scheutzle D, Butler J. 1991. Quantitative determination of trucking industry workers' exposures to diesel exhaust particles. Am Ind Hyg Assoc J 52:529–541.